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(54) Title: INHIBITION OF HIV-1 REPLICATION BY ANTISENSE RNA EXPRESSION

(57) Abstract

Novel antisense sequences to the unspliced or single spliced portions of mRNA transcript from HIV-1 provirus, optionally co-expressed with an inhibitory transdominant mutant HIV-1 protein, are found to be useful in the treatment of HIV-1 infection.

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INHIBITION OF HIV-1 REPLICATION BY ANTISENSE RNA EXPRESSION

NEW VECTORS

This invention relates to inhibition of HIV-1 replication using antisense RNA expression.

HIV-1 infection is believed to be the primary cause of Acquired Immunodeficiency Syndrome (AIDS). HIV-1 is a retrovirus having a genome comprised of two copies of full length RNA. Without intending to be bound by a particular theory, it is hypothesized that the replication of the virus in the CD4+ host cell occurs as follows. When the host cell is infected, the viral genomic RNA is transcribed by reverse transcriptase into double stranded DNA. This double stranded DNA is then integrated into the host cell's chromosome(s). When this double stranded DNA is integrated into the genetic material of the host cell, it is called a provirus. Following activation of the host cell, the provirus is transcribed into RNA in two distinct phases. In the early phase of infection, RNA transcripts of the provirus produced in the nucleus are converted into multiple copies of short sequences by cellular splicing enzymes. These short RNA transcripts encode genes for proteins, e.g., *tat*, which regulate the further transcription, and *rev*, which is thought to mediate the transition into the late phase transcription. This early phase dominates for about 24 hours. About 24 hours after activation of the cell, the transcription moves into the late phase. In late phase transcription, long unspliced RNA transcripts of about 9,200 bases and medium-length single-spliced transcripts of about 4,500 bases move out of the nucleus and into the cytoplasm. These unspliced and single spliced transcripts encode the structural and enzymatic proteins of the virus. These unspliced and single-spliced transcripts include, inter alia, the following regions: *gag*, which encodes the viral core proteins; *pol*, which encodes various enzymes; and *env*, which encodes the two envelope proteins. Figure 1 depicts the HIV-1 genomic structure. It will be noted that there is some overlap in the genes, because certain genes share some base sequences.

The unspliced and single-spliced transcripts are then further spliced, and the resulting mRNA is translated to produce the proteins necessary to make a new virus. The *gag* and *pol* regions are translated to produce the polyproteins gag and gag-pol, which are then cleaved by protease to form the mature proteins found in the virus. The *env* is spliced to generate a subgenomic messenger which encodes for the env polyproteins, which is likewise cleaved to produce the mature envelope proteins. Two strands of the viral RNA are then packaged into a core and surrounded with capsid protein, and the resulting virus is released from the cell together with a portion of the cell membrane.

Various antisense strategies to inhibit HIV-1 infection have been tried, including the use of trans-dominant proteins (**Bevec, D., et al.** 1992. Inhibition of human immunodeficiency virus type 1 replication in human T cells by retroviral-mediated gene transfer of a dominant-negative rev trans-activator. Proc. Natl. Acad. Sci. USA **89:**9870-9874 and **Trono, D., et al.** 1989. HIV-1 gag mutants can dominantly interfere with the replication of the wild-type virus. Cell **59:**113-120), single chain antibodies (**Levy-Mintz, P., et al.** 1996. Intracellular expression of single-chain variable fragments to inhibit early stages of the viral life cycle by targeting human immunodeficiency virus type 1 integrase. J. Virol. **70:**8821-8832.), antisense RNAs (**Chatterjee, S., et al.** 1992. Dual-target inhibition of HIV-1 in vitro by means of adeno-associated virus antisense vector. Science **258:**1485-1488., **Choli, H., et al.** 1994. Inhibition of HIV-1 multiplication in a human CD4+ lymphocytic cell line expressing antisense and sense RNA molecules containing HIV-1 packaging signal and rev response element(s). Antisense Res. and Dev. **4:**19-29, **Joshi, S., et al.** 1991. Inhibition of human immunodeficiency virus type 1 multiplication by antisense and sense RNA expression. J. Virol. **65:**5524-5530, **Kim, J.H., et al.,** 1996. Inhibition of HIV replication by sense and antisense Rev Response Elements in HIV-based retroviral vectors. J. Acquir. Immune Defic. Syndr. **12:**343-351, **Meyer, J., et al.,** 1993. Inhibition of HIV-1 replication by high-copy-number vector expressing antisense RNA for reverse transcriptase. Gene **129:**263-268, **Renneisen, K., et al** 1990. Inhibition

of expression of human immunodeficiency virus-1 in vitro by antibody-targeted liposomes containing antisense RNA to the env region. J. Biol. Chem. **265:**16337-16342 and **Rhodes, A., et al.** 1990. Inhibition of human immunodeficiency virus replication in cell culture by endogenously synthesized antisense RNA. J. Gen. Virol. **71:**1965-1974), RNA decoys (**Lee, T., et al.** 1994. Inhibition of human immunodeficiency virus type 1 in human T cells by a potent Rev-response element decoy consisting of the 13-nucleotide minimal Rev-binding domain. J. Virol. **68:**8254-8264 and **Sullenger, B.A., et al** 1990. Overexpression of TAR sequences renders cells resistant to human immunodeficiency virus replication. Cell **63:** 601-608), and ribozymes (**Ojwang, J.O., et al** 1992. Inhibition of human immunodeficiency virus type 1 expression by a hairpin ribozyme. Proc. Natl. Acad. Sci. USA. **89:** 10802-10806 and **Zhou C., I. Bahner, et al** 1994. Inhibition of HIV-1 in human T lymphocytes by retrovirally transduced anti-*tat* and *rev* hammerhead ribozymes. Gene. **149:**33-39).

The trans-dominant HIV-1 protein RevM10 was first evaluated in a clinical trial using genetically modified peripheral blood lymphocytes (**Woffendin, C et al.** 1996. Expression of a protective gene prolongs survival of T cells in human immunodeficiency virus infected patients. Proc. Natl. Acad. Sci. USA. **93:**2889-2894), although recently a ribozyme (**Leavitt, M.C., et al** 1996. Ex vivo transduction and expansion of CD4+ lymphocytes from HIV+ donors: prelude to a ribozyme gene therapy trial. Gene Ther. **3:**599-606) and a transdominant Rev and antisense TAR based (**Morgan R.A et al** 1996. Clinical protocol: Gene therapy for AIDS using retroviral mediated gene transfer to deliver HIV-1 antisense TAR and transdominant Rev protein genes to syngeneic lymphocytes in HIV-1 infected identical twins. Hum. Genc Ther. **7:**1281-1306.) approach have received RAC and FDA approval.

Intracellular expression of antisense RNAs offers an attractive, alternative gene therapy approach to inhibit HIV-1 replication. Antisense RNAs have been described as very specific and efficient inhibitors in both prokaryotic and eukaryotic systems . Viral replication has been successfully inhibited by addition of in vitro synthesized antisense

oligonucleotides or intracellularly expressed antisense RNAs . Inhibition of HIV-1 replication has been shown previously using antisense RNAs targeted against several viral regulatory (Chatterjee et al 1992, Joshi et al 1991, Kim et al 1996, **Sczakiel, G. et al** 1991. Inhibition of human immunodeficiency virus type 1 replication in human T cells stably expressing antisense RNA. J.Virol. **65:** 468-472 and **Sczakiel, G et al** 1992. Tat- and Rev-directed antisense RNA expression inhibits and abolishes replication of human immunodeficiency virus type 1: a temporal analyses. J. Virol. **66:** 5576-5581) and structural gene products (Choli et al 1994, Gyotoky, et al 1991 , Meyer et al 1993 and Rhodes et al 1990). A few reports described long antisense sequences expressed either intracellularly using retroviral vectors (Choli et al 1994, Gyotoky, et al 1991 and Rhodes et al 1990) or using antibody-targeted liposomal delivery (Renneisen et al). The different inhibition levels observed in these reports may reflect variation in antisense RNA expression levels, or secondary and tertiary RNA structures, which can influence the hybridization kinetics between two complementary RNAs (**Sczakiel, G., M. Homann, and K. Rittner.** 1993 Computer-aided search for effective antisense RNA target sequences of the human immunodeficiency virus type 1. Antisense Res. and Dev. **3:**45-52), influencing the biological activity.

Generally, these efforts have targeted the early phase transcription (e.g., *tat* or *rev* genes) or have targeted RNA processing or initiation of translation in the late phase. Shorter antisense sequences have been favored due to the perceived risk of the antisense sequence folding to form a secondary structure with itself. To date, these efforts have not met with significant success.

It is now surprisingly discovered that the best target for antisense therapy is the full length or single-spliced RNA transcript. Antisense sequences which bind to multiple-spliced transcripts for a gene are less effective, probably because binding to the smaller transcripts results in fewer antisense molecules being available for the binding to the full length or single spliced transcripts. Moreover, longer sequences directed to the full length transcript (e.g., sequences greater than 600 base pairs, preferably greater than 1000 base

pairs) are surprisingly effective and, contrary to the suggestion in the art, do not appear to form undesirable secondary structures.

Hereinafter we present the results of the antiviral activity of sequences complementary to the pol, vif, env genes and 3'LTR in HIV-1 infection experiments using a human $CD4^+$ T cell line (CEM-SS) and peripheral $CD4^+$ T lymphocytes (PBLs). Retroviral vectors are constructed expressing chimeric RNAs containing 1,100 - 1,400 nt long complementary HIV-1 sequences. The most efficient inhibition of HIV-1 replication is observed with an antisense sequence complementary to the HIV-1 *env* gene both in the CEM-SS cell line and in PBLs. This strong antiviral effect is further demonstrated in high inoculation dose infection experiments where reduction of the HIV-1 mRNAs correlates with low level of Gag and Tat protein production indicating that antisense RNA acts early during HIV-1 replication. Comparing the anti-HIV-1 efficacy of the antisense RNAs to the well documented (**Bevec, D.,et al .** 1992. Inhibition of human immunodeficiency virus type 1 replication in human T cells by retroviral-mediated gene transfer of a dominant-negative rev trans-activator. Proc. Natl. Acad. Sci. USA **89:**9870-9874, **Escaich, S., et al** 1995. RevM10-mediated inhibition of HIV-1 replication in chronically infected T cells. Hum. Gene Ther. **6:**625-634, **Malim, M.H., et al.** 1992. Stable expression of transdominant rev protein in human T cells inhibits human immunodefficiency virus replication. J. Exp. Med. **176:**1197-1201 and **Nabel, G.J., et al.** 1995. A molecular genetic intervention for AIDS - effects of a transdominant negative form of Rev. Hum. Gene Ther. **5:**79-92) trans-dominant RevM10 protein demonstrates the potency of the antisense mediated inhibition of HIV-1 replication.

It has further been discovered that antisense sequences to the *gag*, *env*, and *pol*, especially the *env* and *pol* portions of the full length transcript are particularly effective.

The above mentioned antisense constructs are particularly useful for providing gene therapy to patients suffering from HIV-1 infection, e.g., by transducing the HIV-1-susceptable cells of such patients, e.g., CD4+ cells or cells which are progenitors of CD4+ cells, e.g., hematopoietic stem cells (for example CD34+/Thy-1+ cells), with the antisense constructs of the invention, so that the transduced cells and their progeny are resistant to HIV-1 infection.

The antisense constructs of the invention are suitably prepared by incorporating a wild-type HIV-1 gene or gene fragment into a vector in reverse orientation with respect to its promotor so that when the gene is incorporated into the genome of the host cell and transcribed, the opposite strand of the DNA is transcribed, producing a messenger RNA transcript which is complementary to the mRNA from the wild-type gene or gene fragment and will anneal with it to form an inactive RNA-RNA duplex, which is subject to degredation by cellular RNases.

Transduction of the HIV-1 susceptable cells using the antisense vectors can be carried out in vivo or ex vivo, but is suitably carried out ex vivo, by removing blood from the patient, selecting the target cells, inoculating them with a vector containing the antisense construct of the invention, and reintroducing the transduced cells into the body. By natural selection, the transduced HIV-1 resistant cells will replace the native HIV-1 susceptible cells, thereby enabling the patient to overcome the infection and regain immunocompetence. Alternatively, the patient receives non-autologous CD4+ cells or progenitors of CD4+ cells from a compatable donor which cells have been transduced with the antisense construct of the invention.

The invention thus provides:

1. A nucleic acid sequence which, when stably integrated into a human cell, is capable of generating mRNA which anneals e.g., under in vivo conditions, with a mRNA transcript from an HIV-1 provirus encoding env, env and pol or env, pol and gag and which is at least 0.6 kb, preferably at least 1 kb in length, most preferably 1-2 kb, e.g. from 1.1 to 1.5 kb; and which is selected from:

(i) a sequence which is antisense to the 1.4 kb fragment from the Apa1 cleavage site at ca. base 2004 of an HIV-1 provirus to the Pflm1 cleavage site ca. base 3400 of an HIV-1 provirus, e.g. which is antisense to the sequence in figure 1 (SEQ. ID. NO. 1);

(ii) a sequence which is antisense to the 1.2 kb fragment from the Pflim1 cleavage site ca. base 3400 of an HIV-1 provirus to the EcoR1 cleavage site ca. base 4646 of an HIV-1 provirus, e.g. which is antisense to the sequence in figure 2 (SEQ. ID. NO. 2);

(iii) a sequence which is antisense to the 1.3 kb fragment from the ApaL1 cleavage site ca. base 6615 of an HIV-1 provirus to the Bsm1 cleavage site ca. base 8053 of an HIV-1 provirus, e.g., which is antisense to the sequence in figure 3 (SEQ. ID. NO.3) ; and

(iv) a sequence which is at least 80%, preferably at least 90%, more preferably at least 95%, most preferably at least 99%, homologous to a sequence according to (i), (ii), or (iii) and which is capable of generating mRNA which annealss to the same mRNA transcript as that hybridizing to mRNA generated by (i), (ii), or (iii).

It is understood that the nucleic acid described in 1 above will be in RNA for when in a retroviral vector and will be converted to DNA upon incorporation of the provirus into the target cell. It is intended that both the RNA and DNA forms of the constructs are included within the scope of the invention.

The invention further provides

2. A vector comprising an antisense sequence according to 1 above.

The vector may be any vector capable of transducing a human hematopoietic cell, for example, an ecotropic, xenotropic, amphotropic or pseudotyped retroviral vector, an adeno-associated virus (AAV) vector, or an adenovirus (AV) vector. Preferably, the vector is a retroviral vector, preferably a vector characterized in that it has a long terminal repeat sequence (LTR), e.g., a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), or murine embryonic stem cell virus (MESV), or for example, a vector from the pLN series described in Miller and Rosman (1989) BioTechniques 7, pp. 980-986. The antisense sequence replaces the retroviral *gag*, *pol* and/or *env* sequences. The promotor controlling expression of the antisense may be a strong viral promoter, for example MoMLV LTR.

The range of host cells that may be infected by a retrovirus or retroviral vector is generally determined by the viral *env* protein. The recombinant virus generated from a packaging cell can be used to infect virtually any cell type recognized by the *env* protein provided by the packaging cell. Infection results in the integration of the viral genome into the transduced cell and the consequent stable expression of the foreign gene product. The efficiency of infection is also related to the level of expression of the receptor on the target cell. In general, murine ecotropic *env* of MoMLV allows infection of rodent cells, whereas amphotropic *env* allows infection of rodent, avian and some primate cells, including human cells. Xenotropic vector systems utilize murine xenotropic *env*, and also allow infection of human cells. The host range of retroviral vectors may be altered by substituting the *env* protein of the base virus with that of a second virus. The resulting, "pseudotyped" virus has the host range of the virus donating the envelope protein and expressed by the packaging cell line. For example, the G-glycoprotein from vesicular stomatitis virus (VSV-G) may be substituted for the MMLV *env* protein, thereby broadening the host range. Preferably the vector and packaging cell line of the present invention are adapted to be suitable for transduction of human cells. Preferably, the vector is an amphotropic retroviral vector, for example, a vector as described in the examples below.

Optionally, the vector may contain more than one antisense sequence according to 1 above, e.g., two different antisense sequences, for example to pol and env, as described in the examples below.

Preferably, the construct lacks the retroviral *gag, pol* and/or *env* sequences, so that the *gag, pol* and *env* functions must be provided *in trans* by a packaging cell line. Thus, when the vector construct is introduced into the packaging cell, the *gag-pol* and *env* proteins produced by the cell assemble with the vector RNA to produce replication-defective or transducing virions that are secreted into the culture medium. The virus thus produced can infect and integrate into the DNA of the target cell, but generally will not produce infectious viral particles since it is lacking essential viral sequences. The packaging cell line is preferably transfected with separate plasmids encoding *gag-pol* and *env*, so that multiple recombination events are necessary before a replication-competent retrovirus (RCR) can be produced. Suitable retroviral vector packaging cell lines include those based on the murine NIH/3T3 cell line and include PA317 (Miller & Buttimore (1986) *Mol. Cell Biol.* **6**:2895; Miller & Rosman (1989) *BioTechniques* **7**:980), CRIP (Danos & Mulligan (1988) *Proc. Natl Acad Sci USA* **85**:6460), and gp + am12 (Markowitz et al. (1988) *Virology* **167**:400); and also cell lines based on human 293 cells or monkey COS cells, for example ProPak A packaging cells, e.g., as described in Pear et al. (1993) *Proc. Natl. Acad. Sci. USA* 90: 8392-8396; Rigg et al., (1996) *Virology* 218; Finer, et al. (1994) *Blood* 83: 43-50; Landau, et al. (1992) *J. Virol.* 66: 5110-5113. Retroviral vector DNA can be introduced into packaging cells either by stable or transient transfection to produce retroviral vector particles.

The antisense constructs of the invention have the further advantage that they will not interfere with expression of HIV inhibitory proteins, e.g., transdominant mutant proteins corresponding to the early phase short mRNA transcripts, for example mutants of tat or rev. Expression of such transdominant mutant proteins is useful in treating HIV infection because the mutant proteins interfere with the function of the wild-type HIV proteins and so inhibit HIV replication. A transdominant mutant protein of particular interest is RevM10, the use of which is described e.g., in Escaich, et al. Hum. Gene Ther. (1995) 6: 625-634, and

in WO 90/14427. Previously, co-expression of HIV antisense and transdominant mutant proteins was considered impractical because it was expected that the antisense would interfere with expression of the mutant protein. Using the antisense constructs of the invention, co-expression of the antisense with the transdominant mutant protein is not only feasible but provides a synergistic inhibition of the HIV by interfering with the virus at different stages of its replication cycle.

Thus the invention provides in a further embodiment:

3. A retroviral vector according to 2 above (i.e., comprising an antisense sequence according to 1 above) and further comprising a gene for an HIV-1 inhibitory protein, e.g., a gene for a transdominant mutant form of tat or rev, especially the gene for RevM10.

Packaging cell lines comprising the vectors according to 2 or 3 above, e.g, as described above, are also within the scope of the invention.

The invention also provides in a further embodiment:

4. A cellular composition comprising at least one human hematopoietic cell (e.g. CD4+ cell or progenitor of CD4+ cells, e.g., a stem cell, e.g., a CD34+/Thy-1+ cell) stably transduced with an antisense sequence according to 1 above and optionally additionally transduced with a gene for a transdominant mutant form of tat or rev, especially RevM10, e.g., transduced with a vector according to 2 or 3, supra, e.g., for use in a method according to 5 below;

The invention also provides in a further embodiment:

5. A method for treatment of HIV-1 infection in a subject in need thereof comprising

isolating hematopoietic cells (e.g. CD4+ cells or progenitors of CD4+ cells, e.g., stem cells, e.g., CD34+/Thy-1+ cells) from said patient;

transducing said cells with an antisense sequence according to 1 above, and optionally additionally or simultaneously transducing said cells with a gene for an HIV-1 inhibiting transdominant mutant form of tat or rev, especially RevM10, e.g., transducing said cells with a vector according to 2 or 3, supra; and

reintroducing the transduced cells into the patient.

The invention also provides in a further embodiment:

6. The use of an antisense sequence according to 1 above or a vector according to 2 or 3 above in the manufacture of a cellular composition according to 4 above or in a method of treatment according to 5 above.

Figure 1 depicts the sequence of HIV-1 HXB2 strain polymerase gene region 1 (2004-3400 bp) in sense orientation.

Figure 2 depicts the sequence of HIV-1 HXB2 strain polymerase gene region 2 (3400-4650 bp) in sense orientation.

Figure 3 depicts the sequence of the HIV-1 HXB2 strain envelope gene region (6615-8053) in sense orientation.

Figure 4 depict the HIV-1 genomic structure. The position of antisense fragment used for vector construction is also shown. The position of the restriction endonuclease cleavage sites is indicated for each fragment.

Figure 5 depicts the schematic structure of antisense vectors of the examples. The parental vector pLN-1 is described in the publication of A. Dusty Miller and Guy J. Rosman (1989) BioTechniques 7. 980-986. The multicloning site 3' from the Neo gene is used to insert the antisense fragments. The parental vector for the combination vectors pLMTNL is described in : Escaich, S. Kalfoglu, C.; Plavec, I.; et al. Human Gene Therapy 1995. 6. 625-634.

Figure 6 depicts serial deletion of HIV gag sequence. Construction of the deletion fragments is described below. The 1.5 kb Sac I - Bgl II psi-gag fragment (Ψ-gag) is used to generate the deletion construct either by PCR amplification or by restriction digest.

Figure 7 depicts HIV challenge of deletion constructs. The pLN-gag (S) and pLN-gag (AS) construct correspond to the full length 1.5 kb psi-gag fragment in sense or antisense orientation respectively. The pLN-gag-500 is the 5' end of the above fragment

corresponding mostly to the psi (packaging signal) sequence of the HIV-1. The pLN-gag-1000 construct corresponds to the gag region of the 1.5 kb fragment.

Figure 8 depicts the anti-HIV-1 activity of antisense gag deletion fragments as a function of their length; correlation between size and anti-HIV-1 activity is shown on the graph. The p24 production (pg/10E6 cells) versus the length of the fragments in base pairs is plotted on the graph.

Figure 9 depicts HIV-1 challenge of antisense gag and Vif constructs. The full length, 1.5 kb antisense gag (pLN1 Psi-sense and antisense) and the similar size Vif fragment (pLN1 Vif/sense and antisense) are compared.

Figure 10 depicts HIV-1 challenge of gag-pol/AS constructs with high dose of virus (40000 TCID50): The 1.5 kb psi-gag fragment (pLN-gag/AS and S) was compared with the pol-1 fragment (pLM-pol/AS and S).

Figure 11 depicts HIV-1 challenge of antisense pol, env and LTR constructs. CEMSS cells carrying the pol-1 fragment (pLN-pol (AS)/1 and (S)/1) the second antisense pol-2 (pLN Dpol (AS)/2) the envelope (pLN D Env (AS)) and the 3'LTR) pLN D LTR (AS)) fragments are challenged with 400 TCID HIV-1.

Figure 12 depicts the pLN pol1/env antisense vectors, and the effectiveness of pol1(S), pol1(AS), pol1(AS)/env(S), pol(AS)/env(AS) against HIV-1 challenge, the double antisense construct being the most effective.

Figure 13 depicts HIV-1 challenge of combination vectors. The two parental vectors LMTNL with the RevM10 gene and the LAMTNL with ATG less RevM10 gene as a control and the corresponding combination vectors LMTNL-Y and LAMTNL-Y with the full length, 1.5 kb psi-gag sequence in antisense orientation are challenged with 400 TCID50 HIV-1.

Figure 14 depicts pol antisense mediated inhibition of HIV replication in peripheral blood lymphocytes.

Figure 15. A. Structure of the retroviral vectors encoding the antisense sequences. Neo and Lyt2 are used as a selectable marker genes. The antisense sequence together with the marker gene is expressed from the MoMLV LTR promoter. The arrow indicates the antisense orientation of the inserted HIV-1 sequences. **B.** Northern blot analyses of the antisense RNA expression in transduced CEM-SS cells. The recombinant transcripts carrying the antisense sequences are detected using a Neo specific probe. The lower panel indicates the same blot hybridized with a GAPDH specific probe as a internal standard. Lane 1: pLN vector, lane 2: pLN-pol1/AS, lane 3: pLN-pol2/AS, lane 4:pLN-vif/AS, lane 5:pLN-env/AS, lane 6:pLN-3'LTR/AS, lane 7: pLN-pol12/AS vector respectively.

Figure **16.** Inhibition of HIV-1 replication in transduced CEM-SS cells. **A:** CEM-SS cell populations ($1x10^6$ cells/ml) are inoculated with $4x10^2$ $TCID_{50}$/ml of HIV-1 HXB3 strain. **B:** Increasing HIV-1 dose, $4x10^4$ $TCID_{50}$/ml infection of transduced CEM-SS cell populations. The culture supernatants are tested for p24 antigen production by ELISA. experiments are done in duplicates.

Figure **17.** Evaluation of anti-HIV-1 efficacy of vectors encoding different length complementary pol sequences. **A.** Anti-HIV-1 efficacy of pol1_deletion constructs. CEM-SS cells expressing the 1,400 nt pol1 and 790 nt pol antisense and the sense pol1 constructs are infected with $4x10^3$ $TCID_{50}$/ml of HIV-1 HXB3 strain. **B.** CEM-SS cells expressing the 1,400 nt pol1 and the 2,600 nt pol12 antisense sequences are infected with $4x10^3$ $TCID_{50}$/ml HIV-1 HXB3 strain. The corresponding sense constructs are used as a control.

Figure **18.** Antisense RNA expression and inhibition of HIV-1 replication in transduced PBLs. **A.** Total cellular RNA is isolated from activated, $CD4^+$ enriched PBLs transduced with pL-Lyt-pol1/AS, pL-Lyt2/pol1/S, pL-Lyt-env/AS, pL-Lyt2/env/S vectors and selected for Lyt2 expression. The antisense transcripts are analyzed on Northern blot using a radiolabeled Lyt2 specific probe. A GAPDH specific probe is used to monitor the amount of RNA loaded. Lane 1:pL-Lyt2-pol1/AS, Lane 2:pL-Lyt2-pol1/S, Lane 3: pL-Lyt2-env/AS, Lane 4: pL-Lyt-env/S, Lane 5:pL-Lyt2-pol1/AS. **B.** Transduced and $CD4^+$, $Lyt2^+$ selected

PBLs are activated with allogenic feeder cells and infected with the clinical HIV-1 isolate JR-CSF. $5x10^4$ cells are inoculated in triplicate, and p24 antigen production is determined.

Figure **19.** Comparison of trans-dominant RevM10 and intracellularly expressed vif, pol1 and env antisense RNAs in high inoculation dose HIV-1 infection experiments. CEM-SS cells ($1x10^6$/ml) are inoculated with $1x10^5$ $TCID_{50}$/ml of HIV-1 HXB3 and viral replication is monitored by measuring p24 antigen production in the culture supernatant.

Figure **20.** Detection of HIV-1, antisense and RevM10 transcripts in CEM-SS cells inoculated with $1x10^5$ $TCID_{50}$/ml HIV-1, HXB3 strain. Total cellular RNA is isolated from CEM-SS cells at day 4, day 6 and day 8 post infection. The HIV specific transcripts are analyzed on Northern blot using a radiolabeled TAR specific oligonucleotide probe. Expression of the antisense or RevM10 transcripts is determined using a Neo or a Rev specific probe respectively. A GAPDH specific probe is used to monitor the amount of RNA loaded. Lane 1: RevM10, Lane 2: DRevM10, Lane 3: pLN(vector control), Lane 4: pLN-vif/AS, Lane 5:pLN-pol1/AS, Lane 6:pLN- env/AS. Panel A: Day 4., Panel B: day 6, Panel C: Day 8.

Figure **21.** Analyses of intracellular p24 and Tat expression in HIV-1 infected CEM-SS cells. **A.** Intracellular p24 expression is measured at day 8 post infection. The mean fluorescence intensity reflects the relative intracellular p24 expression level. **B.** Detection of Tat protein in transduced and HIV-1 infected CEM-SS cells. Aliquots of infected CEM-SS cells at day 8 post infection are fixed in methanol, stained with Tat specific antibody and analyzed by FACScan.

EXAMPLE 1:

Construction of retroviral vectors carrying antisense HIV-1 sequences

Retroviral vector constructs with different antisense HIV-1 sequences are generated as follows using as parental vector pLN, described in A. Dusty Miller and Guy J. Rosman (1989) BioTechniques 7. 980-986.

a) pLN-gag/AS vector: The 1420 bp Sac I-Bgl II (675 bp-2095 bp) fragment is isolated from the HXB-2 strain of HIV-1 and inserted as a blunt end fragment in antisense orientation into the blunt ended Hind III site of the pLN-1 vector. Orientation of the fragment is determined by restriction digest with Cla I.

b) 3' Deletion pLN-gag/AS vectors: Serial deletion fragments from the 1420 bp Sac I-Bgl II (675 bp-2095 bp) fragment are generated by PCR amplification. The 5' end of the fragments are fixed using the GAGCTCTCTCGACGCAGGACT (SEQ. ID. NO. 4) primer at position 675 bp-695 bp). The primers at the 3' end were the following; primer 3.6: position 1897-1900
GTAGGATCCGTTACTTGGCTCATTGCTTCA (SEQ. ID. NO. 5); primer 3.5: position 1677-1700.
CACGGATCCGAGTTTTATAGAACCGGTCTAC (SEQ. ID. NO. 6); primer 3.4: position 1479-1500
GTAGGATCCACTGCTATGTCACTTCCCCTTGG (SEQ. ID. NO. 7); primer 3.3 position 1280-1300,
GTAGGATCCACATGGGTATCACTTCTGGGCTG (SEQ. ID. NO. 8); primer 3.2 position 1079-1100,

GTAGGATCCTCTATCTTGTCTAAAGCTTCCTTG (SEQ. ID. NO. 9); primer 3.1 position 884-904,
GTAGGATCCCCTGCTTGCCCATACTATATG (SEQ. ID. NO. 10). The PCR fragments with Bam HI and blunt end are cloned into the Bam HI - Hpa I site of the pLN vector. The generated fragments are approximately 1200 bp, 1000 bp, 800 bp, 600 bp, 400 bp 200 bp in length.

c) Removal of the psi sequence from the gag fragment: The 1420 bp SAC I-Bgl II (675 bp-2095 bp) fragment is digested with Pvu II resctriction endonuclease which removes 494 bp corresponding to the psi packaging signal form the 5' end of the fragment. The resulting fragment (gag 500/AS and gag 1000/AS) is cloned as a blunt end fragment into the Hind II of pLN vector.

d) pLN-Vif/AS vector: The 1100 bp Eco RI-Eco Ri fragment (4646-5742) from the HXB-2 strain of HIV-1 corresponding Vif-Vpr gene of the virus is inserted into the Hind II site of pLN vector in antisense orientation.

e) pLN-pol1/AS vector: The 1480 bp Apa I-Pflm I fragment (2005-3485) from the HXB-2 strain of HIV-1 corresponding to the 5' end of the Pol gene of the virus is inserted into the Hind II site of pLN vector in antisense orientation.

f) pLN-pol2/AS vector: The 1250 bp Pflm I - Eco RI fragment (3485-4646) from the HXB-2 strain of HIV-1 corresponding to the 3' end of the Pol gene of the virus is inserted into the Hind II site of pLN vector in antisense orientation.

g) pLN-env/AS vector: The 1440 bp Apa LI - Bsm I fragment (6615-8053) from the HXB-2 strain of HIV-1 corresponding to intronic region of the Env gene of the virus is inserted into the Hind II site of pLN vector in antisense orientation.

h) pLN-pol1(AS)-env(AS) vector: The pol1 fragment of e) is inserted 5' to the env construct of g), both in antisense orientation in the HindII site of the pLN vector (fig. 12).

i) pLN3' LTR/AS vector: the 1260 bp Bam HI-Hind III fragment (8474-9615) from the HXB-2 strain of HIV-1 corresponding to the 3' LTR of the virus is inserted into the Xho I site of pLN vector in antisense orientation.

j) The retroviral vector pLN-pol12/AS with the full length pol sequence is constructed by inserting the 2,642 bp ApaI-EcoRI fragment into the pLN vector in reverse orientation. For the sense control vectors pLN-pol1/S and pLN-pol12/S the 1,400 bp ApaI-PflmI and 2,642 bp ApaI-EcoRI pol fragments are cloned in the sense orientation into the pLN vector. The pLN-790pol/AS vector is constructed by inserting the 790 bp BglII-NsiI subfragment of the pol gene into the XhoI site of the pLN vector. Retroviral vectors (pLLyt2-pol1/AS, pLLyt2-pol1/S, pLLyt2-env/AS and pLLyt2-env/S) are constructed by replacing the *Neo* gene with the truncated mouse CD8 (Lyt2) cell surface marker (**Forestell, S.P., et al** 1997. Novel retroviral packaging cell lines: complementary tropism and improved vector production for efficient gene transfer. Gene Ther. **4**:19-28) and used for the primary T cell HIV-infection experiments.

k) Combination vectors: The LMTNL and the LΔMTNL vectors carrying the transdominant RevM10 gene and its ATG-less form (ΔM) (Escaich, S.; Kalfoglou, C.; Lavec, I.;et. al. Human Gene Therapy 1995. 6 625-634) are digested with Cla I and the 1200 bp Cla I-Bgl II fragment from HXB-2 strain of HIV-1, corresponding to the Gag gene region, is inserted as an antisense fragment.

l) Retroviral vector production: 10 ug of retroviral DNA is transfected into the ecotropic BOSC packaging line using the CaPO4 transfection protocol. The transient ecotropic viral

supernatant is used to transduce the amphotropic PA 317 packaging cell line. Since the pLN vectors carry the Neo gene, the cells are selected on G418. After selection the stable cells pools are analysed by Northern blot for the antisense RNA expression. Viral supernatants from the selected PA317 cell lines carrying the appropriate retroviral constructs are collected, analysed for transducing viral titer, and used to transduce the human CD4+ T cells line CEMSS. GP47 could be used instead of BOSC as the packaging line (**Rigg , R.J., et al** 1996. A novel human amphotropic packaging cell line: high titer, complement resistance, and improved safety.Virology. **218:** 290-295). Supernatant from the GP47 packaging cell lines is used to transduce the amphotropic ProPakA cell line (**Rigg , R.J., et al .** 1996) by spinoculation as described previously (**Forestell, S.P et al .** 1997. Novel retroviral packaging cell lines: complementary tropism and improved vector production for efficient gene transfer. Gene Ther. **4:**19-28). Retroviral end-point titers are determined on NIH3T3 cells after drug selection (800 mg/ml G418) and transduction efficacy of the Lyt2 vectors (**Forestell, S.P et al .** 1997) is measured by FACS analysis.

m) Target cell transduction: The human CD4+ T cell line CEM SS cells (2x 10^6 cells) are transduced with the amphotropic viral supernatants carrying the antisense vector constructs in 5 ml DMEM + 10 FCS + 8 ug/ml polybrene for 4-6 hours. 48 hours later the cells are selected on 400 ug/ml G418. After G418 selection (7-10 days) the resistant cell are expanded, the antisense RNA expression is analysed by Northern blot. The selected CEM SS cell pools are also analysed for the presence of the CD4 cell surface marker.

EXAMPLE 2:

HIV-1 challenge of CEM clones or pools

The resistance of transduced CEM cells to HIV replication and to cytopathic effects of the virus is determined as follows:

Cells are subjected to HIV-1 infection (HXB3) in vitro. Antiviral effect is measured by cell viability, levels of p24 Ag produced in the supernatant, and levels of CD4 expression at the cell surface. Infection is measured by PCR for HIV sequences. In addition to the clones to be challenged, CEMss containing a vector control are submitted to infection by HIV-1.

Day -1: Prior to challenge the clones are tested for CD4 expression by FACS analysis.

Day 0:

1. Count the cell
2. Spin down $2x10^6$ CEM cells 5 min at 1200 rpm
3. Pour off the supernatant from the cells
4. Dilute virus stock in culture medium to 4000 or 400 $TCID_{50/ml}$ (medium: RPMI 1640, 10% CCS, Peni 100 U/ml, Strepto 100 mg/ml)
5. Resuspend the cell pellet ($2x10^6$ cells) in 2 ml of the virus dilution, (or 2 ml of media for the non infected control)
6. Incubate on rotator (low speed 18 rpm) for 2 hours at RT
7. Spin down the cells
8. Aspirate carefully the virus suspension
9. Wash the cells twice in 7 ml medium, by centifugation at 1200 rpm for 10 min
10. Resuspend the cells in 10 ml media (CEM at $2x10^5$/ml final concentration)
11. Incubate at 37°c, 5% CO_2 for 4 days

Day 4:

12. Analysis: - cell count
    - 1 ml of centrifuged supernatant for p24 titration, freeze at -70°C
13. Passage the cells: dilution to $2x10^5$/ml final in fresh medium

Day 8:

14. Analysis at day 8:

- cell count
- 1 ml of centrifuged supernatant for p24 titration, freeze at -70°C
- take 10^6 cells for CD4 staining (optional)
- lyse $2x10^6$ cells in 400 ul for DNA PCR, store at -20°C (optional)
- RNA extraction from $4x10^6$ cells using RNazol, store at -70°C

(optional)

15. Passage the cells: dilution to $2x10^5$/ml final in fresh medium

16. Cells are passaged every 4-5 days to be maintained in log phase growth until day 16 or until the controls are dead. For each passage, cells are counted and supernatant is frozen.

EXAMPLE 3: Detection of intracellular Tat and p24

Transduced CEM-SS cells expressing RevM10 and antisense HIV-1 sequences are inoculated with $1x10^5$ $TCID_{50}/10^6$ cells/ml of HIV-1. At day 4, day 6 and day 8, cells are removed from the culture, washed and resuspended in cold PBS and fixed in ice cold methanol for 30 min. The fixed cells are stained with a FITC-conjugated anti-p24 monoclonal antibody (Coulter KC57) for intracellular p24 detection, include p24, with mouse anti-Tat IgG1 antibody (Repligen) for intracellular Tat detection as described earlier (**Rigg, R.J., et al** 1995. Detection of intracellular HIV-1 Rev protein by flow cytometry. J. Immun. Methods. **188:**187-195). The samples are analyzed using a Becton-Dickinson FACScan.

EXAMPLE 4: **Detection of antisense RNA in cells.**

Total cellular RNA from CEM-SS cells and from activated PBLs is extracted with RNAzol (Cinna/Biotecx). 10 mg RNA is fractionated on 1.2% agarose/formaldehyde gels, transferred to Hybond N membrane (Amersham), and hybridized in Rapid-hyb buffer

(Amersham). Oligonucleotides (100 ng) are radiolabeled with terminal transferase (Boehringer MA), using a-^{32}P-dATP to a specific activity of $3x10^8$ cpm/mg. DNA fragments are labeled by random priming (Boehringer MA). The membranes are hybridized with the labeled probe (5 x 10^6 cpm/ml) at 65 °C for 1 hour and ished with 1xSSC, 0.1% SDS at 65°C, and exposed on X-ray film or analyzed on a PhosphorImager (Molecular Dynamics).

EXAMPLE 5:

Pol Antisense-mediated inhibition of HIV-1 replication in PBLs

Transduction and HIV-1 infection of human PBLs: PBLs are isolated from healthy donors buffy coats by gradient centrifugation. Enriched CD4+ cells are obtained by labeling bulk PBL with biotinylated αCD8+ and αCD19+ antibodies followed by depletion with streptavidin conjugated magnetic beads (Dynabeads M-280, Dynal A.S., Norway). The enriched CD4+ PBLs are stimulated with phytohemagglutinin (PHA, 5 μg/ml) on γ-irradiated allogenic feeder cells for 72 hours in Iscove's modified DMEM medium. PBLs ($2x10^6$) are transduced by spinoculation in the presence of Polybrene (8 μg/ml). After 48 hours, cells are analysed for CD4+ and Lyt2+ expression by flow cytometry using anti-CD4-FITC and anti-CD8-PE conjugated monoclonal antibodies. Lyt2+ expressing PBLs are again enriched by magnetic bead selection. After the first enrichment, PBLs are expanded, and the CD4+/Lyt2+ cells are isolated using fluorescence-activated cell sorting (FACS, Beckton-Dickinson, Vantage). After the second enrichment, greater than 90% of the cell population is CD4+ and Lyt2+. Primary CD4+ T-cells ($5x10^4$) are inoculated with 600 $TCID_{50}$/ml HIV-1 JR-CSF (5) in quadruplicate 4 days after the last restimulation of the cells. Half of the culture supernatant is exchanged daily for 9 days supernatants are stored at -70°C, and p24 Ag is determined by ELISA. Viable cells are counted by trypan blue exclusion 7 days after inoculation.

EXAMPLE 6:

Inhibition of HIV-1 replication in _CEM-SS cells

To compare the efficacy of the antisense sequences, transduced CEM-SS cells expressing complementary transcripts are infected with $4x10^2$ $TCID_{50}$/ml of the HIV-1 HXB3 virus. HIV-1 replication is monitored by measuring p24 antigen levels in the culture supernatant by ELISA. As negative control, a vector encoding the pol sequence in sense orientation (pLN-pol/S) is used. Fig. 16.A shows the relative efficacy of the different antisense sequences at low HIV-1 inoculation dose. CEM-SS cells expressing the env antisense RNA showed almost complete suppression of HIV-1 replication, releasing 50 pg of $p24/10^6$ cells at day 18 post-inoculation. We have observed 3.0 log_{10} reduction of p24 antigen production with the pol1 and pol2 antisense sequences and 1.0 log_{10} reduction with the vif antisense sequence. The 3'LTR antisense construct is indistinguishable from the control vector, which correlates with the low expression level of antisense transcript observed by Northern blot (Fig. 15 B.). In the following experiment, we increased the HIV-1 inoculation dose 100-fold to $4x10^4$ $TCID_{50}$/ml and tested only the pol1, pol2, vif and env antisense constructs (Fig. 16B.). Overall, the onset of HIV- replication is much earlier and the replication kinetics are much faster than in the low MOI experiment. At day 10, the control CEM-SS cells(pLN-pol1/S) released high levels of p24 antigen in the culture supernatants ($2x10^6$ pg $p24/10^6$ cells). However, at this time point HIV-1 virus replication is still substantially inhibited in all antisense CEM-SS cultures relative to control, Although, HIV replication levels are higher than in the previous experiment, intracellular env expression is again the most potent inhibitor (3.0 log_{10} reduction) followed by pol1 and pol2 (2.0 log_{10} reduction) and the antisense vif sequence is the least potent antiviral inhibitor (1.0 log_{10} reduction). Similar results are observed when antisense RNA expressing CEM-SS cells are infected with the less cytopathic SF2 HIV-1 strain (data not shown).

EXAMPLE 7:

Effect of antisense RNA length on HIV inhibition.

To confirm that our observation is not specific to the Y-gag antisense RNA, a vector encoding a shorter pol antisense fragments as described in Materials and Methods is constructed. The antiviral potency of the 790 nt long antisense pol fragment and the 1,400 nt pol1 fragment is compared at $4x10^3$ $TCID_{50}$/ml of HIV-1 HXB3. An approximately 50% decrease in anti-HIV-1 efficacy with the shorter pol1 sequence relative to the 1,400 nt pol1 fragment is observed as shown in Fig. 17.A. This experiment provide further evidence that the length of the retrovirally expressed antisense RNA is an important factor for antiviral efficacy.

A vector encoding an antisense transcript of the complete *pol* gene reading frame is also generated to address the question whether increasing the antisense RNA length beyond 1,400 nt results in increased antiviral efficacy. Figure 17.B demonstrates that the 1,400 nt pol1 antisense sequence is as efficient in blocking HIV-1 replication as the 2,600 nt pol12 antisense RNA. Since both pol1 and pol2 antisense RNA yield comparable levels of inhibition, this experiment suggests that other factors in addition to expression level and transcript length may influence the efficacy of antisense RNA.

EXAMPLE 8:

Comparison of anti-HIV-1 efficacy of RevM10 and antisense RNAs.

The antiviral potency of antisense vif, pol1, and env sequences at a high HIV-1 inoculation dose with RevM10, the trans-dominant form of the HIV-1 Rev protein is compared. RevM10 acts post-transcriptionally, preventing the transport of full length HIV-1 transcripts from the nucleus to the cytoplasm. In order to test at which step the antisense RNA interferes with the HIV-1 life cycle, the effect of RevM10 and antisense RNA on HIV-1 RNA steady-state levels as well as on structural (p24 gag) and regulatory (Tat) protein expression is analyzed. Polyclonal CEM-SS cell populations expressing RevM10, DRevM10 (**Plavec, I., et al** 1997. High transdominant RevM10 protein levels are required to inhibit HIV-1 replication in cell lines and primary T cells: implication for gene therapy of AIDS. Gene Ther. **4**:128-139) and antisense vif, pol and env sequences are inoculated

with $1x10^5$ $TCID_{50}/10^6$ cells HIV-1 HXB3 (MOI: 0.1). The analyses of secreted p24 antigen release into the cell supernatant indicate the rapid progression of viral replication in the control cultures (pLN and DRevM10), as well as in the RevM10 and vif/AS cell populations (Fig. 19.). In contrast, 2.0 orders of magnitude lower p24 production is observed with the pol/AS and env/AS RNA expressing cells lines. Total RNA samples isolated from HIV-1 infected cells at day 4, day 6 and day 8 post infection are analyzed. Northern blot analyses of day 4 samples shows low levels of HIV-1 transcripts in all cultures (Fig.20.A.). At this time point, the steady-state expression levels of all recombinant transcripts are comparable. At day 6 post infection (Fig. 20.B.), the control vector (lane 3) and DRevM10 (lane 2) transduced cells express high steady-state levels of HIV-1 transcripts. The RevM10 (lane 1) and vif/AS (lane 4) vector transduced cells express 3-to 5-fold less than the respective control cell populations, and the pol/AS (lane 5) and env/AS (lane 6) vector transduced cells still express very low HIV-1 RNA levels (Fig.20.B.). At this time point there are still comparable amount of recombinant transcript present in all cultures (lower panel). Analyses of the day 8 RNA samples (Fig.20.C.) demonstrated degradation and decreased amounts of all 3 RNA transcripts analyzed (HIV-1, vector transcripts and GAPDH) in the control cell populations, probably due to the massive HIV-1 induced cell death in these cultures. High levels of HIV-1 RNA are detected in the RevM10 and vif/AS expressing cells, increased about 5-fold in the pol/AS expressing cells, but is still very low in the env/AS RNA expressing cells. At the same time point, we also analyzed the intracellular p24 Gag and Tat protein levels in the infected cell population. FACS analysis of day 8 samples demonstrate that 27 % of the pol/AS and only 5 % of the env/AS RNA expressing cells express detectable amount of p24 Gag protein (Fig.21.A.), which correlates with the observed low HIV transcript levels. At this time point, almost 100 % of the CEM-SS cells expressing the RevM10 gene or vif/AS RNA are positive for intracellular p24 Gag protein, although the vif/AS population produced lower p24 antigen levels (mean fluorescence intensity 135).

Measuring the intracellular Tat protein levels gave similar results, although the sensitivity of this assay is lower than for the p24 Gag protein detection. Fig.21.B demonstrates that only 3-5 % of antisense pol and env RNA expressing cells produce detectable Tat protein, which can explain the observed low overall HIV transcript levels.

The HIV-inhibitory effects of the vectors are depicted in figures 7 through 21. Vectors containing longer antisense fragments are more effective inhibitors, as are vectors containing antisense to the gag, pol, and/or the env regions. Combination vectors containing revM10 plus an antisense construct are more effective than vectors containing revM10 or antisense alone.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Organic Compounds

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1396 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GGGTAGAGAC AACAACTCCC CCTCAGAAGC AGGAGCCGAT AGACAAGGAA CTGTATCCTT 240

TAACTTCCCT CAGGTCACTC TTTGGCAACG ACCCCTCGTC ACAATAAAGA TAGGGGGGCA 300

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GCCAGGAAGA TGGAAACCAA AAATGATAGG GGGAATTGGA GGTTTTATCA AAGTAAGACA 420

GTATGATCAG ATACTCATAG AAATCTGTGG ACATAAAGCT ATAGGTACAG TATTAGTAGG 480

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ATTCCAAAGT AGCATGACAA AAATCTTAGA GCCTTTTAGA AAACAAAATC CAGACATAGT 1080

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AACAAAAATA GAGGAGCTGA GACAACATCT GTTGAGGTGG GGACTTACCA CACCAGACAA 1200

AAAACATCAG AAAGAACCTC CATTCCTTTG GATGGGTTAT GAACTCCATC CTGATAAATG 1260

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1250 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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AAAGTTGTCA CCCTAACTGA CACAACAAAT CAGAAGACTG AGTTACAAGC AATTTATCTA 600

GCTTTGCAGG ATTCGGGATT AGAAGTAAAC ATAGTAACAG ACTCACAATA TGCATTAGGA 660

ATCATTCAAG CACAACCAGA TCAAAGTGAA TCAGAGTTAG TCAATCAAAT AATAGAGCAG 720

TTAATAAAAA AGGAAAAGGT CTATCTGGCA TGGGTACCAG CACACAAAGG AATTGGAGGA 780

AATGAACAAG TAGATAAATT AGTCAGTGCT GGAATCAGGA AAGTACTATT TTTAGATGGA 840

ATAGATAAGG CCCAAGATGA ACATGAGAAA TATCACAGTA ATTGGAGAGC AATGGCTAGT 900

GATTTTAACC TGCCACCTGT AGTAGCAAAA GAAATAGTAG CCAGCTGTGA TAAATGTCAG 960

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TGTACACATT TAGAAGGAAA AGTTATCCTG GTAGCAGTTC ATGTAGCCAG TGGATATATA 1080

GAAGCAGAAG TTATTCCAGC AGAAACAGGG CAGGAAACAG CATATTTTCT TTTAAAATTA 1140

GCAGGAAGAT GGCCAGTAAA AACAATACAT ACTGACAATG GCAGCAATTT CACCGGTGCT 1200

ACGGTTAGGG CCGCCTGTTG GTGGGCGGGA ATCAAGCAGG AATTTGGAAT 1250

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1391 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CACTGATTTG AAGAATGATA CTAATACCAA TAGTAGTAGC GGGAGAATGA TAATGGAGAA 60

AGGAGAGATA AAAAACTGCT CTTTCAATAT CAGCACAAGC ATAAGAGGTA AGGTGCAGAA 120

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TAGCTTGACA AGTTGTAACA CCTCAGTCAT TACACAGGCC TGTCCAAAGG TATCCTTTGA 240

GCCAATTCCC ATACATTATT GTGCCCCGGC TGGTTTTGCG ATTCTAAAAT GTAATAATAA 300

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TGTAGAAATT AATTGTACAA GACCCAACAA CAATACAAGA AAAAGAATCC GTATCCAGAG 540

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TGTAACGCAC AGTTTTAATT GTGGAGGGGA ATTTTTCTAC TGTAATTCAA CACAACTGTT 780

TAATAGTACT TGGTTTAATA GTACTTGGAG TACTGAAGGG TCAAATAACA CTGAAGGAAG 840

TGACACAATC ACCCTCCCAT GCAGAATAAA ACAAATTATA AACATGTGGC AGAAAGTAGG 900

AAAAGCAATG TATGCCCCTC CCATCAGTGG ACAAATTAGA TGTTCATCAA ATATTACAGG 960

GCTGCTATTA ACAAGAGATG GTGGTAATAG CAACAATGAG TCCGAGATCT TCAGACTTGG 1020

AGGAGGAGAT ATGAGGGACA ATTGGAGAAG TGAATTATAT AAATATAAAG TAGTAAAAAT 1080

TGAACCATTA GGAGTAGCAC CCACCAAGGC AAAGAGAAGA GTGGTGCAGA GAGAAAAAAG 1140

AGCAGTGGGA ATAGGAGCTT TGTTCCTTGG GTTCTTGGGA GCAGCAGGAA GCACTATGGG 1200

CGCAGCCTCA ATGACGCTGA CGGTACAGGC CAGACAATTA TTGTCTGGTA TAGTGCAGCA 1260

GCAGAACAAT TTGCTGAGGG CTATTGAGGC GCAACAGCAT CTGTTGCAAC TCACAGTCTG 1320

GGGCATCAAG CAGCTCCAAG CAAGAATCCT AGCTGTGGAA AGATACCTAA AGGATCAACA 1380

GCTCCTAGCA S 1391

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GAGCTCTCTC GACGCAGGAC T 21

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GTAGGATCCG TTACTTGGCT CATTGCTTCA 30

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CACGGATCCG AGTTTTATAG AACCGGTCTA C 31

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GTAGGATCCA CTGCTATGTC ACTTCCCCTT GG 32

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTAGGATCCA CATGGGTATC ACTTCTGGGC TG 32

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GTAGGATCCT CTATCTTGTC TAAAGCTTCC TTG 33

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTAGGATCCC CTGCTTGCCC ATACTATATG 30

CLAIMS

1. A nucleic acid sequence which, when stably integrated into a human cell, is capable of generating mRNA which anneals with a mRNA transcript from an HIV-1 provirus encoding env, env and pol or env, pol and gag and which is selected from:

(i) a sequence which is antisense to the 1.4 kb fragment from the Apa1 cleavage site at ca. base 2004 of an HIV-1 provirus to the Pflm1 cleavage site ca. base 3400 of an HIV-1 provirus;

(ii) a sequence which is antisense to the 1.2 kb fragment from the Pflim1 cleavage site ca. base 3400 of an HIV-1 provirus to the EcoR1 cleavage site ca. base 4646 of an HIV-1 provirus;

(iii) a sequence which is antisense to the 1.3 kb fragment from the ApaL1 cleavage site ca. base 6615 of an HIV-1 provirus to the Bsm1 cleavage site ca. base 8053 of an HIV-1 provirus; and

(iv) a sequence which is at least 80% homologous to a sequence according to (i), (ii), or (iii) and which is capable of generating mRNA which anneals to the same mRNA transcript as that annealing to mRNA generated by (i), (ii), or (iii).

2. A nucleic acid squence according to claim 1 which is antisense to the sequence depicted in Figure 1, 2, or 3.

3. A retroviral vector comprising at least one nucleic acid sequence according to claim 1 or 2.

4. A retroviral vector according to claim 3 further comprising at least one gene for an HIV inhibitory protein.

5. A retroviral vector according to claim 4 wherein the HIV inhibitory protein is RevM10.

6. A cellular composition comprising at least one human hematopoietic cell stably transduced with an antisense sequence according to claim 1 or 2 above and optionally additionally stably transduced with a gene for an HIV inhibitory protein.

7. The cellular composition according to claim 6 wherein the human hematopoietic cell is a hematopoietic stem cell, and the HIV inhibitory protein is RevM10.

8. A method for treatment of HIV-1 infection in a subject in need thereof comprising
isolating hematopoietic cells from said patient;
transducing said cells using a vector according to any of claims 3, 4 or 5; and
reintroducing the transduced cells into the patient.

9. The use of an antisense sequence according to claim 1 or 2 or a vector according to claim 3, 4 or 5 in the manufacture of a cellular composition according to claim 6 or 7 or in a method of treatment according to claim 8.

10. All novel compounds, processes and utilities substantially as described herein, particularly with reference to the examples.

FIGURE 1

Sequence of HIV-1 HXB2 strain polymerase gene region 1 (2004-3400 bp)

Sense orientation:

GGGCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAATGA
AAGATTGTACTGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCTACAAG
GGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAG
AGAGCTTCAGGTCTGGGGTAGAGACAACAACTCCCCCTCAGAAGCAGGAGCCGAT
AGACAAGGAACTGTATCCTTTAACTTCCCTCAGGTCACTCTTTGGCAACGACCCCT
CGTCACAATAAAGATAGGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGAGCA
GATGATACAGTATTAGAAGAAATGAGTTTGCCAGGAAGATGGAAACCAAAAATGA
TAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGA
AATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC
ATAATTGGAAGAAATCTGTTGACTCAGATTGGTTGCACTTTAAATTTTCCCATTAG
CCCTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAAGTT
AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAG
AGATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATAC
TCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGAT
TTCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGAATAC
CACATCCCGCAGGGTTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGTGA
TGCATATTTTTCAGTTCCCTTAGATGAAGACTTCAGGAAGTATACTGCATTTACCAT
ACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCA
CAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAG
AGCCTTTTAGAAAACAAAATCCAGACATAGTTATCTATCAATACATGGATGATTTG
TATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAGCTGA
GACAACATCTGTTGAGGTGGGGACTTACCACACCAGACAAAAAACATCAGAAAGA
ACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGC
CTATAGTGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTAGT
GGGGAAATTGAATTGGGCAAGTCAGATTTACCCAGGGATTAAAGTAAGGCAATTA
TGTAAACTCCTTAGA

FIGURE 2

Sequence of HIV-1 HXB2 strain polymerase gene region 2 (3400-4650 bp).

Sense orientation

GGAACCAAAGCACTAACAGAAGTAATACCACTAACAGAAGAAGCAGAGCTAG
AACTGGCAGAAAACAGAGAGATTCTAAAAGAACCAGTACATGGAGTGTATTAT
GACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAAT
GGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAATAT
GCAAGAATGAGGGGTGCCCACACTAATGATGTAAAACAATTAACAGAGGCAG
TGCAAAAAATAACCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTT
AAACTGCCCATACAAAAGGAAACATGGGAAACATGGTGGACAGAGTATTGGC
AAGCCACCTGGATTCCTGAGTGGGAGTTTGTTAATACCCCTCCCTTAGTGAAAT
TATGGTACCAGTTAGAGAAAGAACCCATAGTAGGAGCAGAAACCTTCTATGTA
GATGGGGCAGCTAACAGGGAGACTAAATTAGGAAAAGCAGGATATGTTACTA
ATAGAGGAAGACAAAAAGTTGTCACCCTAACTGACACAACAAATCAGAAGAC
TGAGTTACAAGCAATTTATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACAT
AGTAACAGACTCACAATATGCATTAGGAATCATTCAAGCACAACCAGATCAAA
GTGAATCAGAGTTAGTCAATCAAATAATAGAGCAGTTAATAAAAAAGGAAAA
GGTCTATCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAA
GTAGATAAATTAGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAAT
AGATAAGGCCCAAGATGAACATGAGAAATATCACAGTAATTGGAGAGCAATG
GCTAGTGATTTTAACCTGCCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTG
TGATAAATGTCAGCTAAAAGGAGAAGCCATGCATGGACAAGTAGACTGTAGTC
CAGGAATATGGCAACTAGATTGTACACATTTAGAAGGAAAAGTTATCCTGGTA
GCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTTATTCCAGCAGAAAC
AGGGCAGGAAACAGCATATTTTCTTTTAAAATTAGCAGGAAGATGGCCAGTAA
AAACAATACATACTGACAATGGCAGCAATTTCACCGGTGCTACGGTTAGGGCC
GCCTGTTGGTGGGCGGGAATCAAGCAGGAATTTGGAAT

FIGURE 3

Sequence of the HIV-1 HXB2 strain envelope gene region (6615-8053)

Sense orientation:

CACTGATTTGAAGAATGATACTAATACCAATAGTAGTAGCGGGAGAATGATAA
TGGAGAAAGGAGAGATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGA
GGTAAGGTGCAGAAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATA
GATAATGATACTACCAGCTATAGCTTGACAAGTTGTAACACCTCAGTCATTACA
CAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCG
GCTGGTTTTGCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACC
ATGTACAAATGTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTAT
CAACTCAACTGCTGTTAAATGGCAGTCTAGCAGAAGAAGAGGTAGTAATTAGA
TCTGTCAATTTCACGGACAATGCTAAAACCATAATAGTACAGCTGAACACATCT
GTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAAAGAATCCGTAT
CCAGAGAGGACCAGGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATG
AGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATAACACTTTAAAACA
GATAGATAGCAAATTAAGAGAACAATTCGGAAATAATAAAACAATAATCTTTA
AGCAATCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAATTGTGGA
GGGGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGTACTTGGTTTAAT
AGTACTTGGAGTACTGAAGGGTCAAATAACACTGAAGGAAGTGACACAATCAC
CCTCCCATGCAGAATAAAACAAATTATAAACATGTGGCAGAAAGTAGGAAAA
GCAATGTATGCCCCTCCCATCAGTGGACAAATTAGATGTTCATCAAATATTACA
GGGCTGCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCCGAGATCTT
CAGACTTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAA
TATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGA
GAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCT
TGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGA
CGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG
CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCAT
CAAGCAGCTCCAAGCAAGAATCCTAGCTGTGGAAAGATACCTAAAGGATCAAC
AGCTCCTAG

FIGURE 4

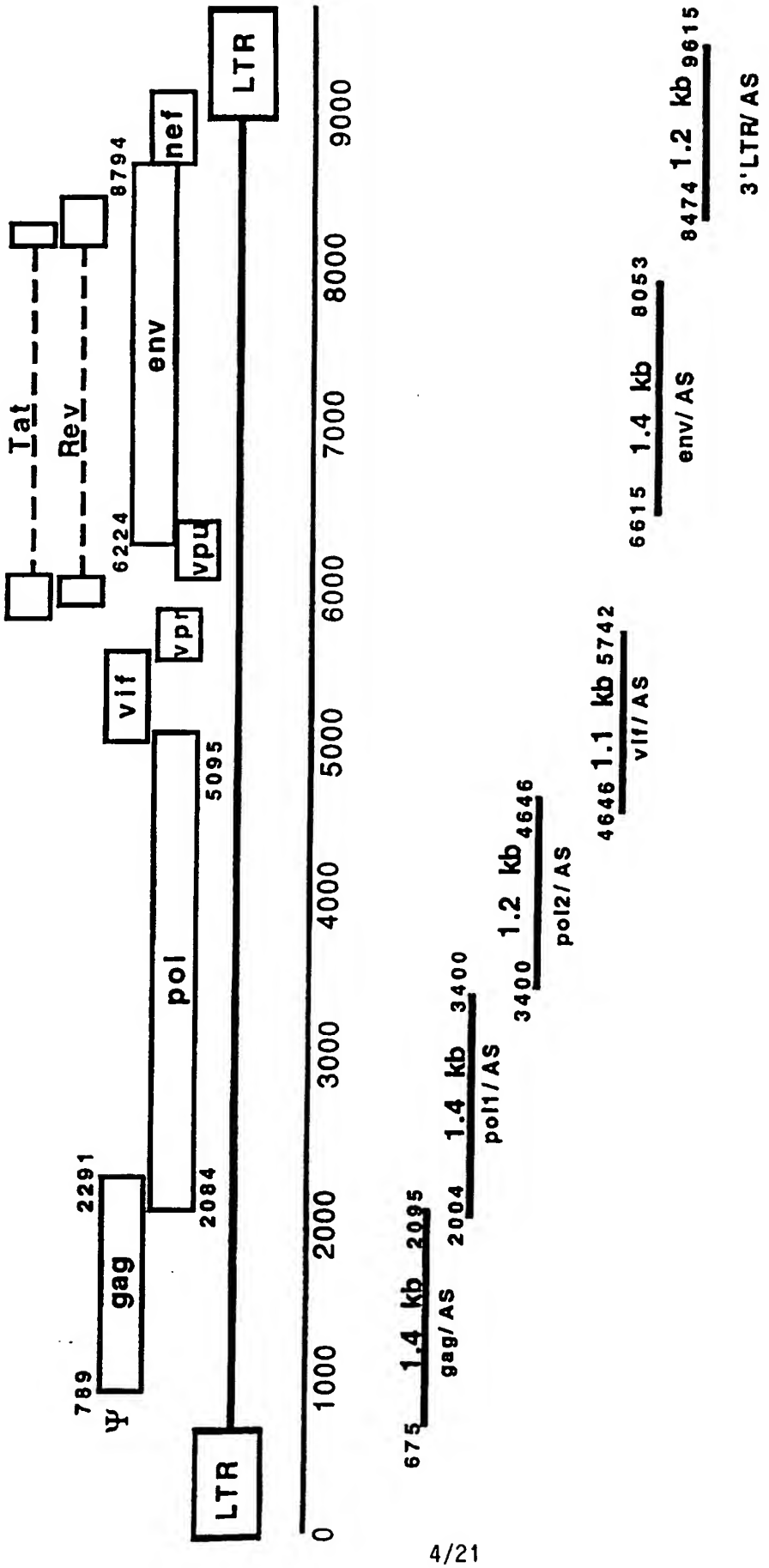
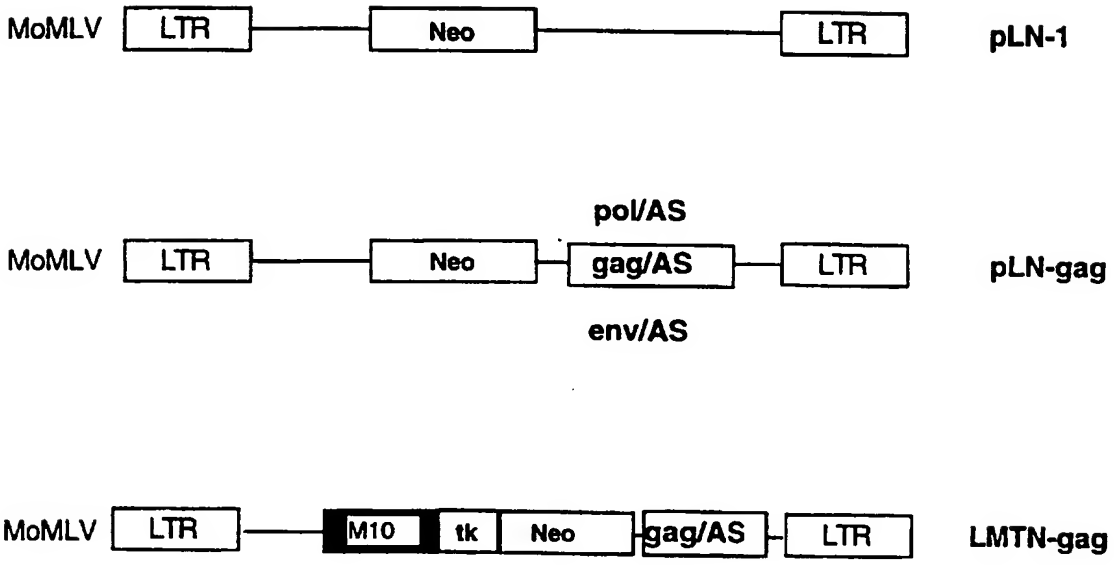
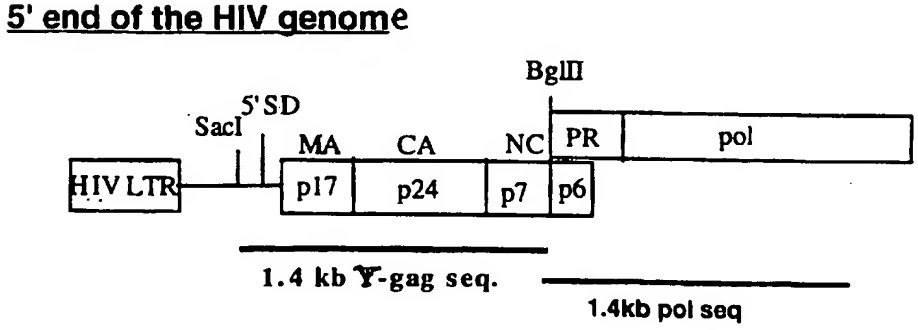

LTR
nef
env
vpu
Tat
Rev
vpr
vif
pol
gag
Ψ
LTR
789
2291
2084
5095
6224
8794
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
675
1.4 kb 2095
gag/AS
2004
1.4 kb 3400
pol1/AS
3400
1.2 kb 4646
pol2/AS
4646
1.1 kb 5742
vif/AS
6615
1.4 kb 8053
env/AS
8474
1.2 kb 9615
3'LTR/AS

Figure 5

Antisense vectors





Serial deletion of HIV gag sequence

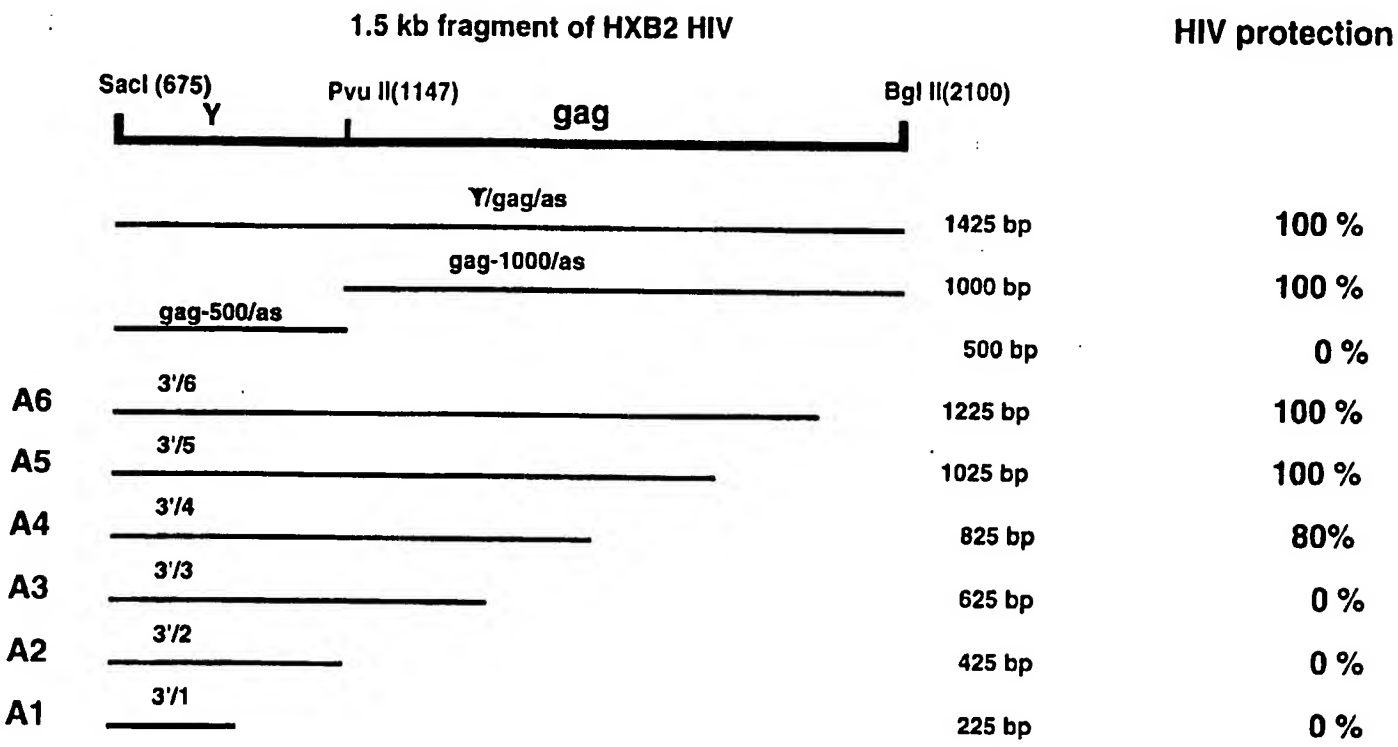


Figure 6

FIGURE 7

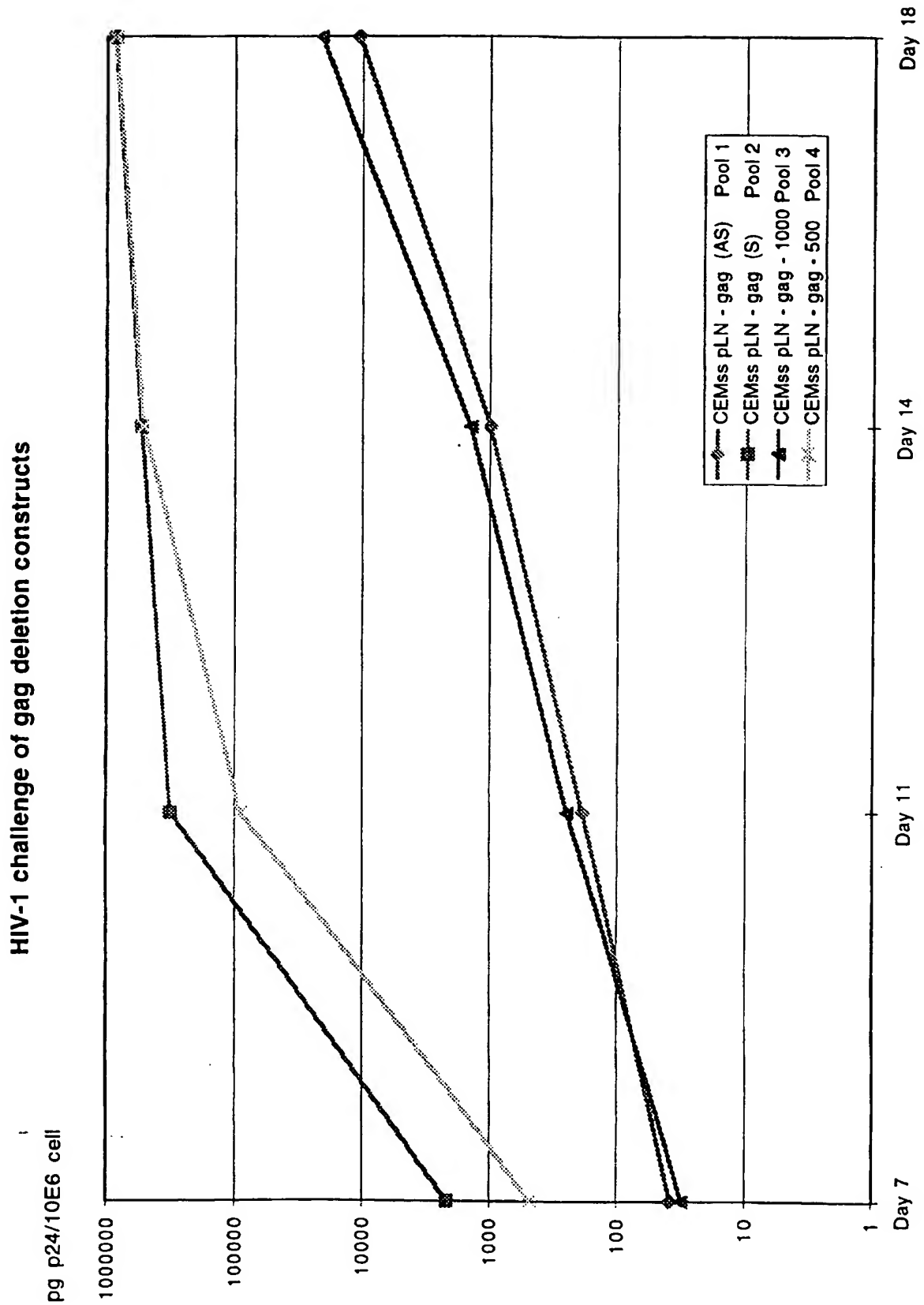

HIV-1 challenge of gag deletion constructs
pg p24/10E6 cell
1000000
100000
10000
1000
100
10
1
Day 7
Day 11
Day 14
Day 18
CEMss pLN - gag (AS) Pool 1
CEMss pLN - gag (S) Pool 2
CEMss pLN - gag - 1000 Pool 3
CEMss pLN - gag - 500 Pool 4

FIGURE 8

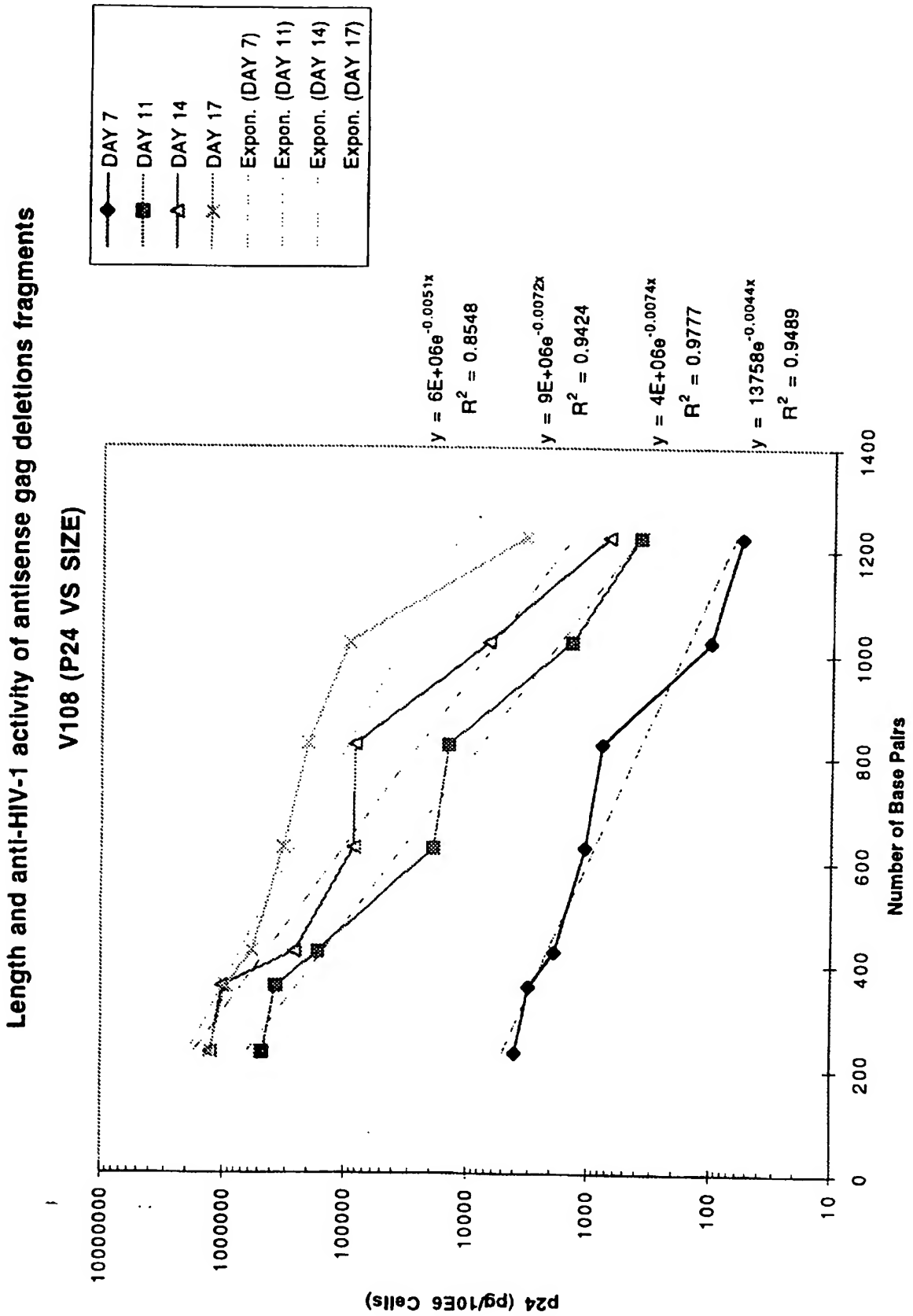

Length and anti-HIV-1 activity of antisense gag deletions fragments
V108 (P24 VS SIZE)
DAY 7
DAY 11
DAY 14
DAY 17
Expon. (DAY 7)
Expon. (DAY 11)
Expon. (DAY 14)
Expon. (DAY 17)
y = 6E+06e^-0.0051x
R^2 = 0.8548
y = 9E+06e^-0.0072x
R^2 = 0.9424
y = 4E+06e^-0.0074x
R^2 = 0.9777
y = 13758e^-0.0044x
R^2 = 0.9489
p24 (pg/10E6 Cells)
10000000
1000000
100000
10000
1000
100
10
0
200
400
600
800
1000
1200
1400
Number of Base Pairs

FIGURE 9

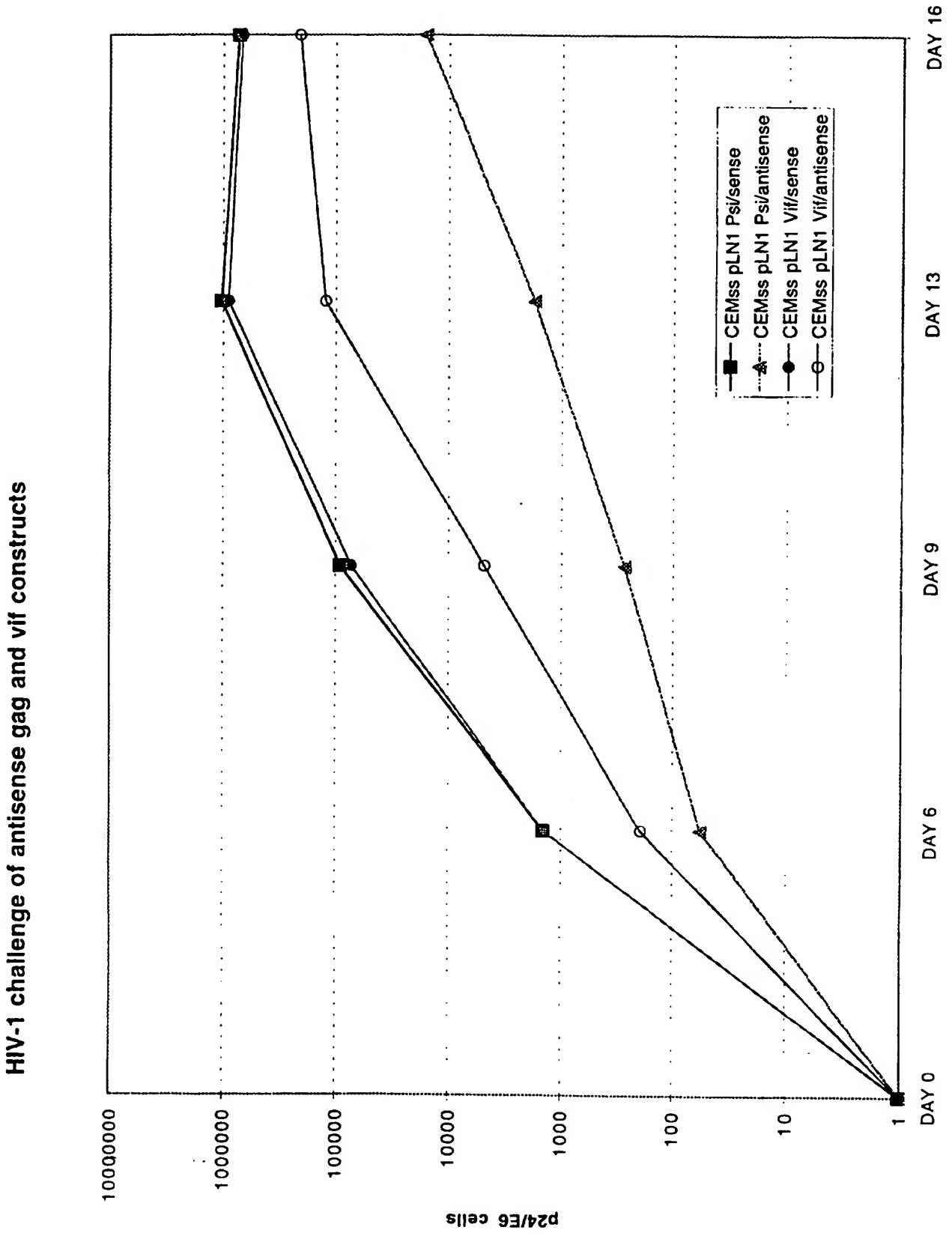

HIV-1 challenge of antisense gag and vif constructs
p24/E6 cells
10000000
1000000
100000
10000
1000
100
10
1
DAY 0
DAY 6
DAY 9
DAY 13
DAY 16
CEMss pLN1 Psi/sense
CEMss pLN1 Psi/antisense
CEMss pLN1 Vif/sense
CEMss pLN1 Vif/antisense

FIGURE 10

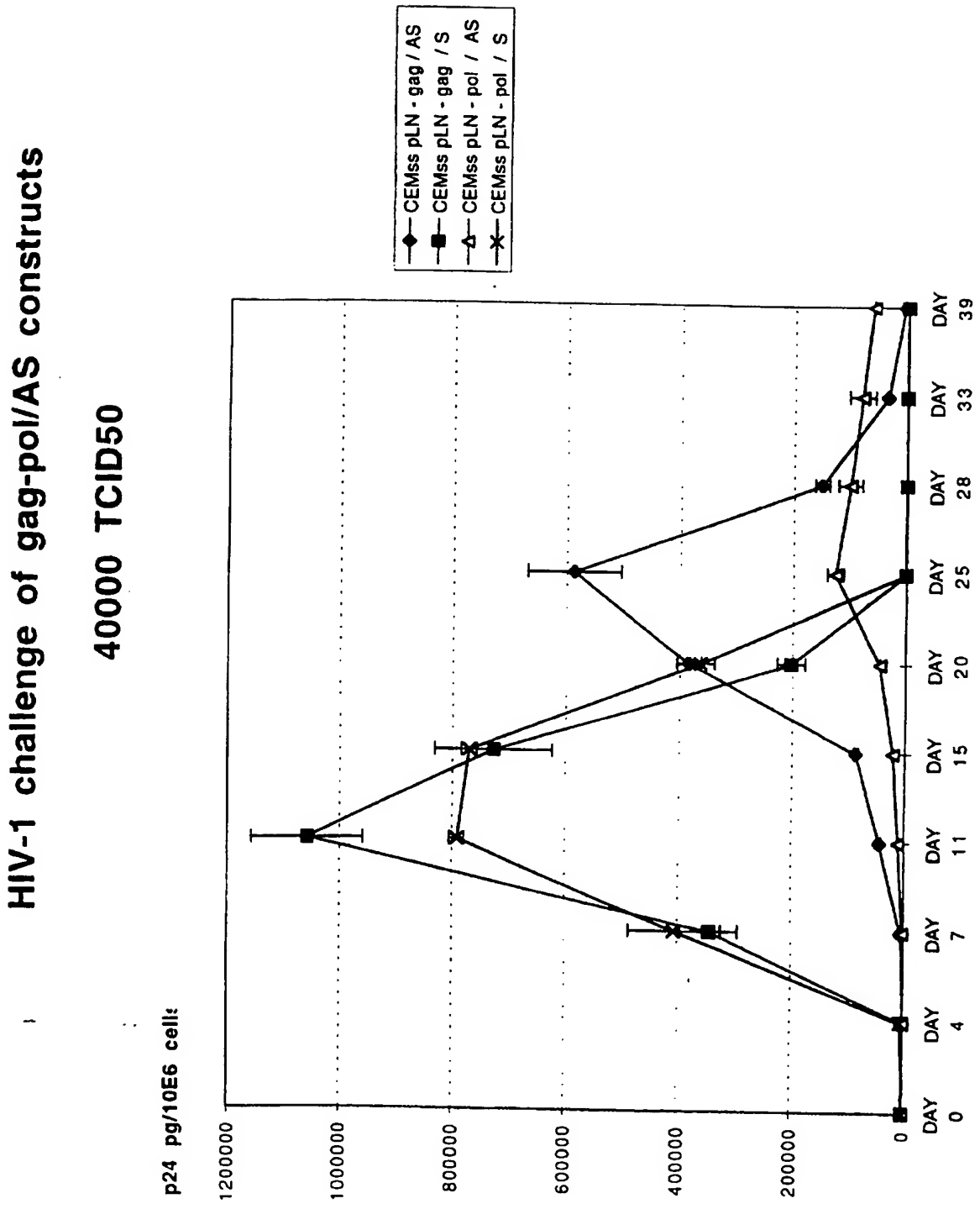

HIV-1 challenge of gag-pol/AS constructs
40000 TCID50
p24 pg/10E6 cells
CEMss pLN - gag / AS
CEMss pLN - gag / S
CEMss pLN - pol / AS
CEMss pLN - pol / S
1200000
1000000
800000
600000
400000
200000
0
DAY 0
DAY 4
DAY 7
DAY 11
DAY 15
DAY 20
DAY 25
DAY 28
DAY 33
DAY 39

FIGURE 11

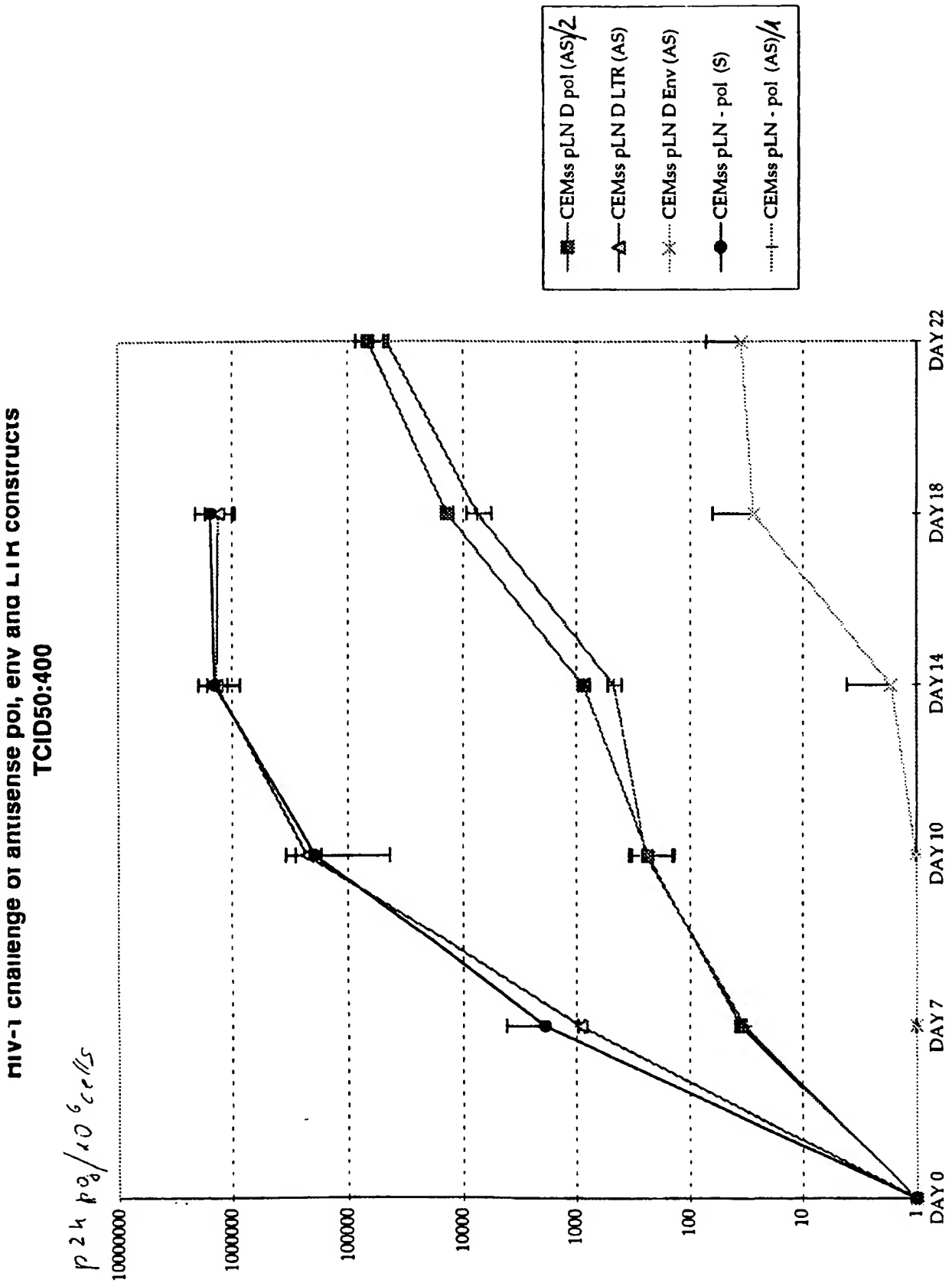
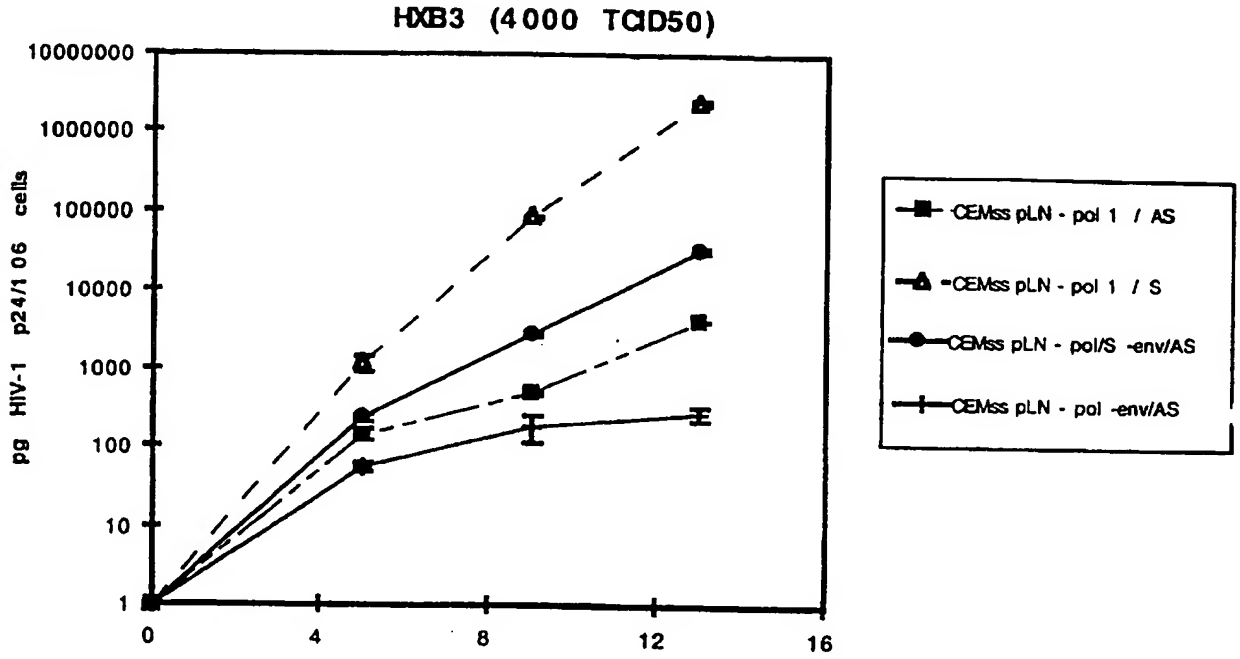

HIV-1 challenge of antisense pol, env and LTR constructs
TCID50:400
p24 pg/ml
10000000
1000000
100000
10000
1000
100
10
1
DAY 0
DAY 7
DAY 10
DAY 14
DAY 18
DAY 22
CEMss pLN D pol (AS)/2
CEMss pLN D LTR (AS)
CEMss pLN D Env (AS)
CEMss pLN - pol (S)
CEMss pLN - pol (AS)/4

FIGURE 12

HIV-1 challenge of pol1-env antisense combination constructs



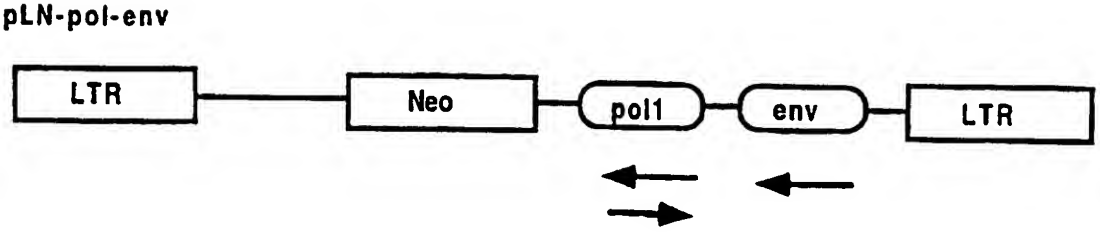


FIGURE 13

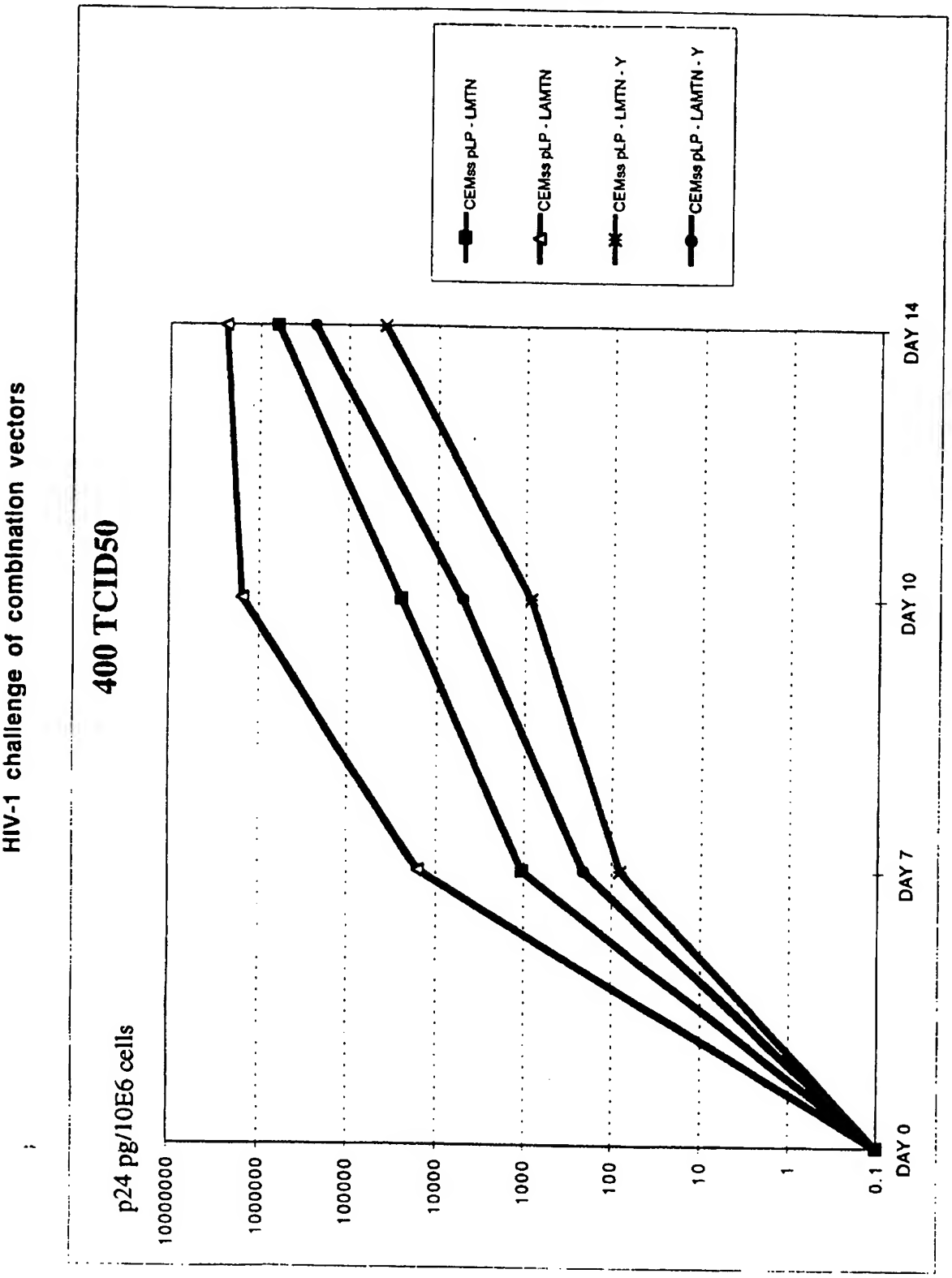

HIV-1 challenge of combination vectors
400 TCID50
p24 pg/10E6 cells
10000000
1000000
100000
10000
1000
100
10
1
0.1
DAY 0
DAY 7
DAY 10
DAY 14
CEMss pLP - LMTN
CEMss pLP - LAMTN
CEMss pLP - LMTN - Y
CEMss pLP - LAMTN - Y

FIGURE 14

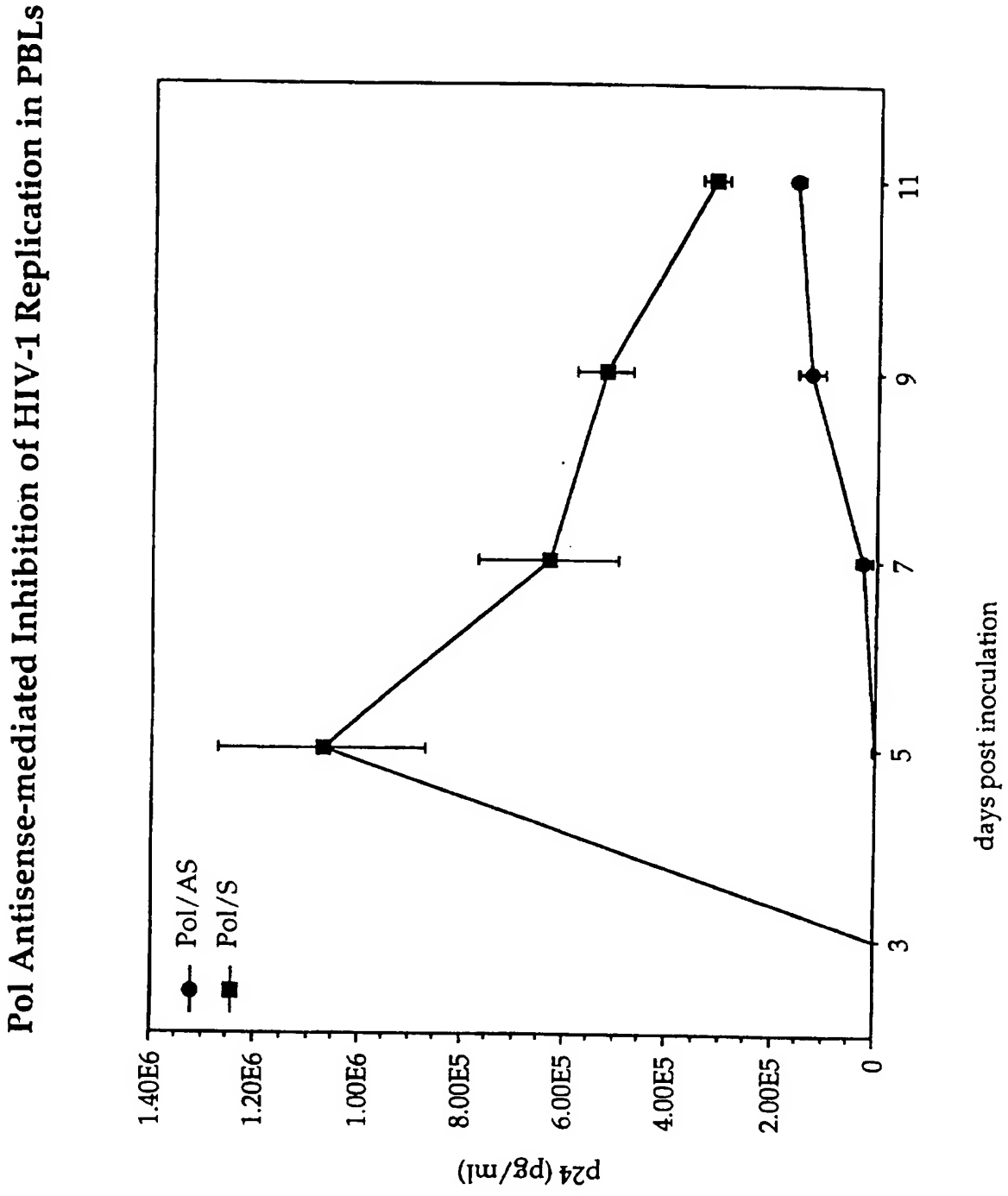
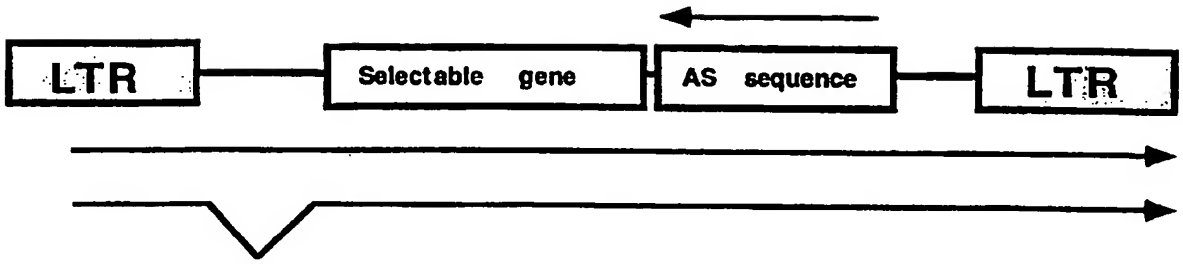

Pol Antisense-mediated Inhibition of HIV-1 Replication in PBLs
Pol/AS
Pol/S
p24 (pg/ml)
1.40E6
1.20E6
1.00E6
8.00E5
6.00E5
4.00E5
2.00E5
0
3
5
7
9
11
days post inoculation

FIGURE 15

A



B

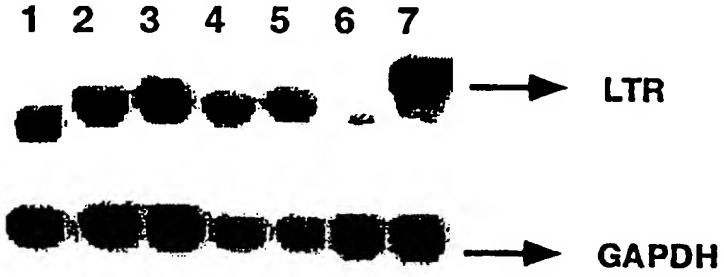
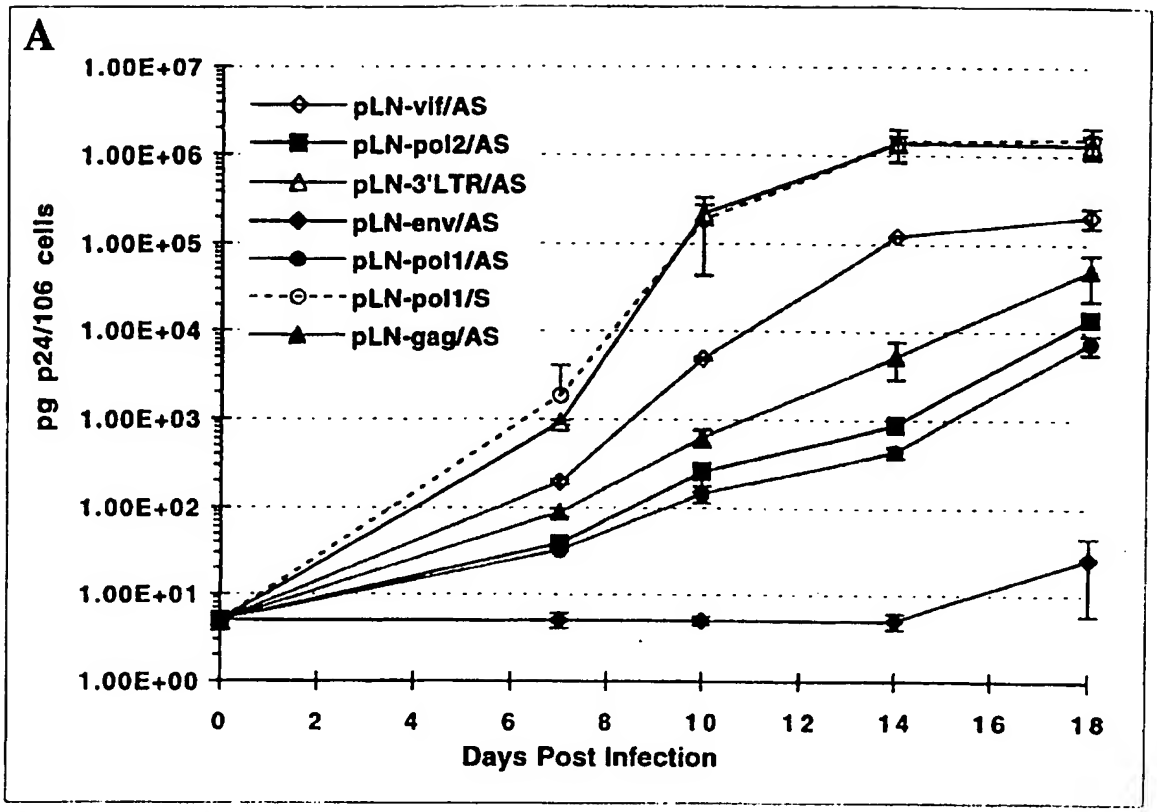


FIGURE 16


A
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
pg p24/106 cells
pLN-vif/AS
pLN-pol2/AS
pLN-3'LTR/AS
pLN-env/AS
pLN-pol1/AS
pLN-pol1/S
pLN-gag/AS
0 2 4 6 8 10 12 14 16 18
Days Post Infection

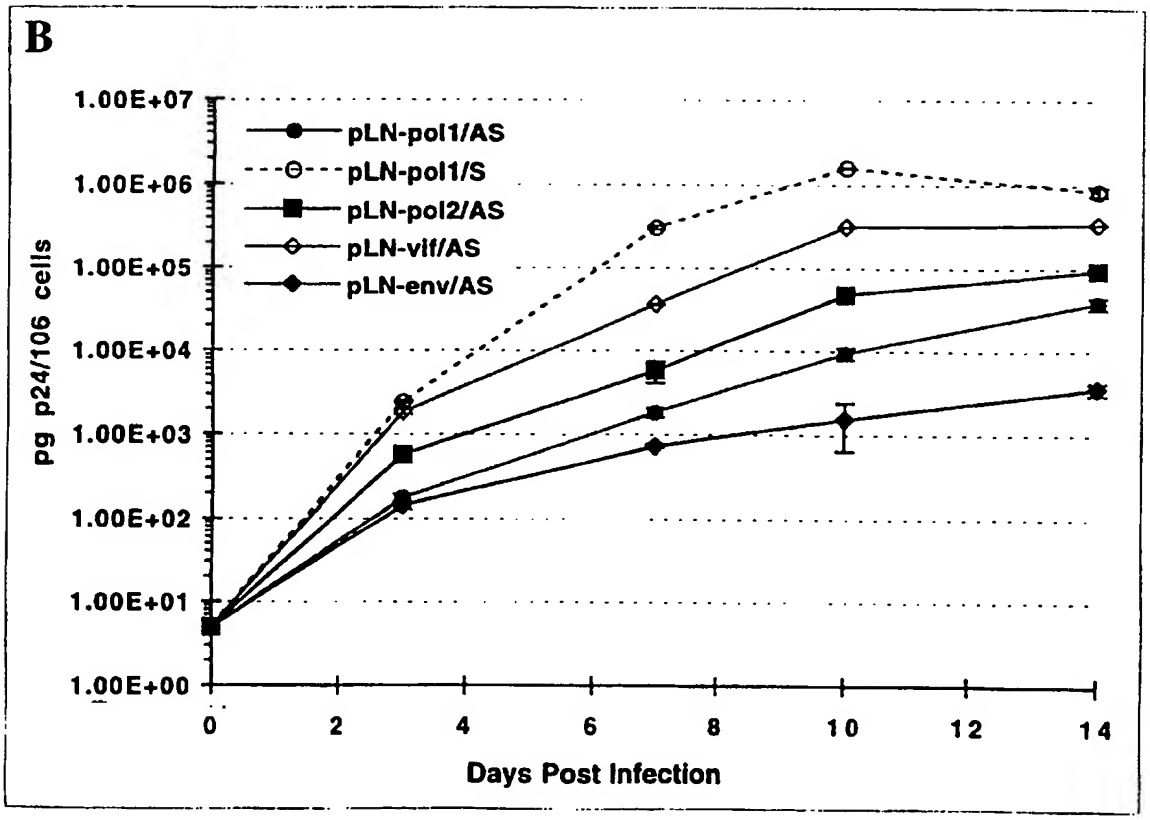
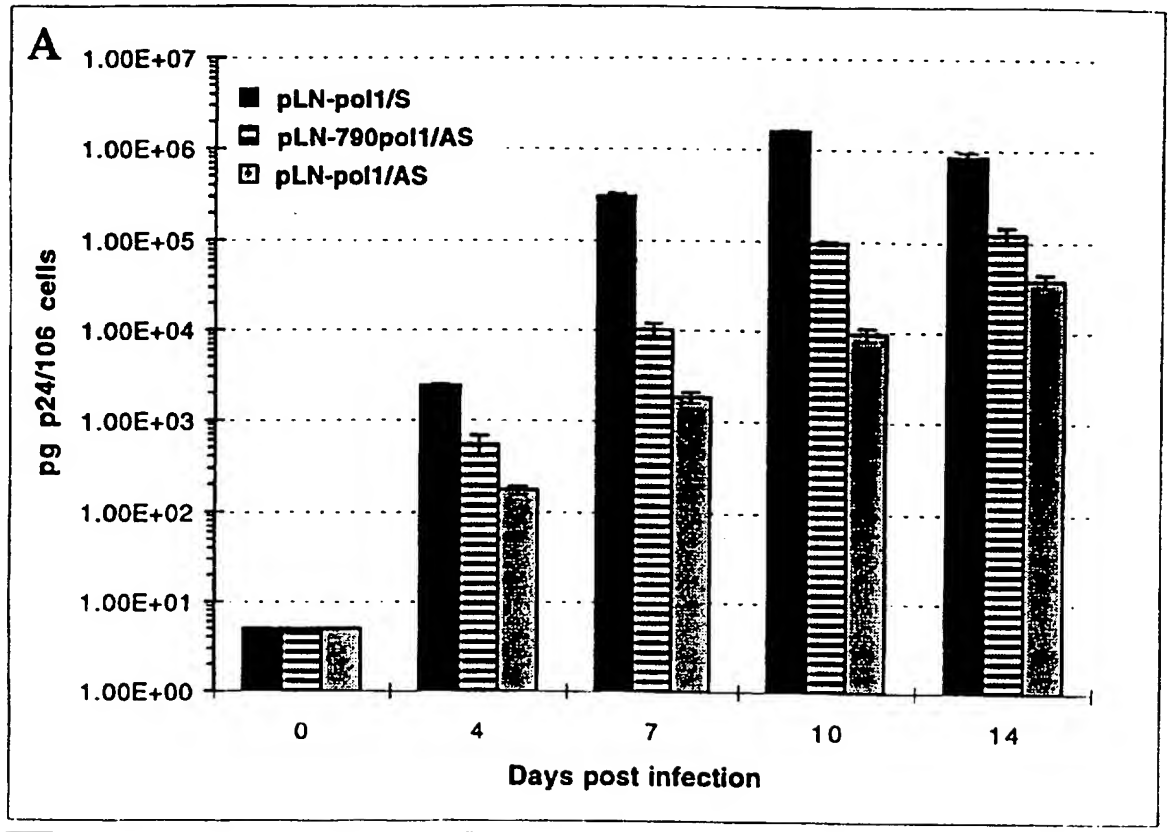

B
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
pg p24/106 cells
pLN-pol1/AS
pLN-pol1/S
pLN-pol2/AS
pLN-vif/AS
pLN-env/AS
0 2 4 6 8 10 12 14
Days Post Infection

FIGURE 17


A
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
pg p24/106 cells
pLN-pol1/S
pLN-790pol1/AS
pLN-pol1/AS
0
4
7
10
14
Days post infection

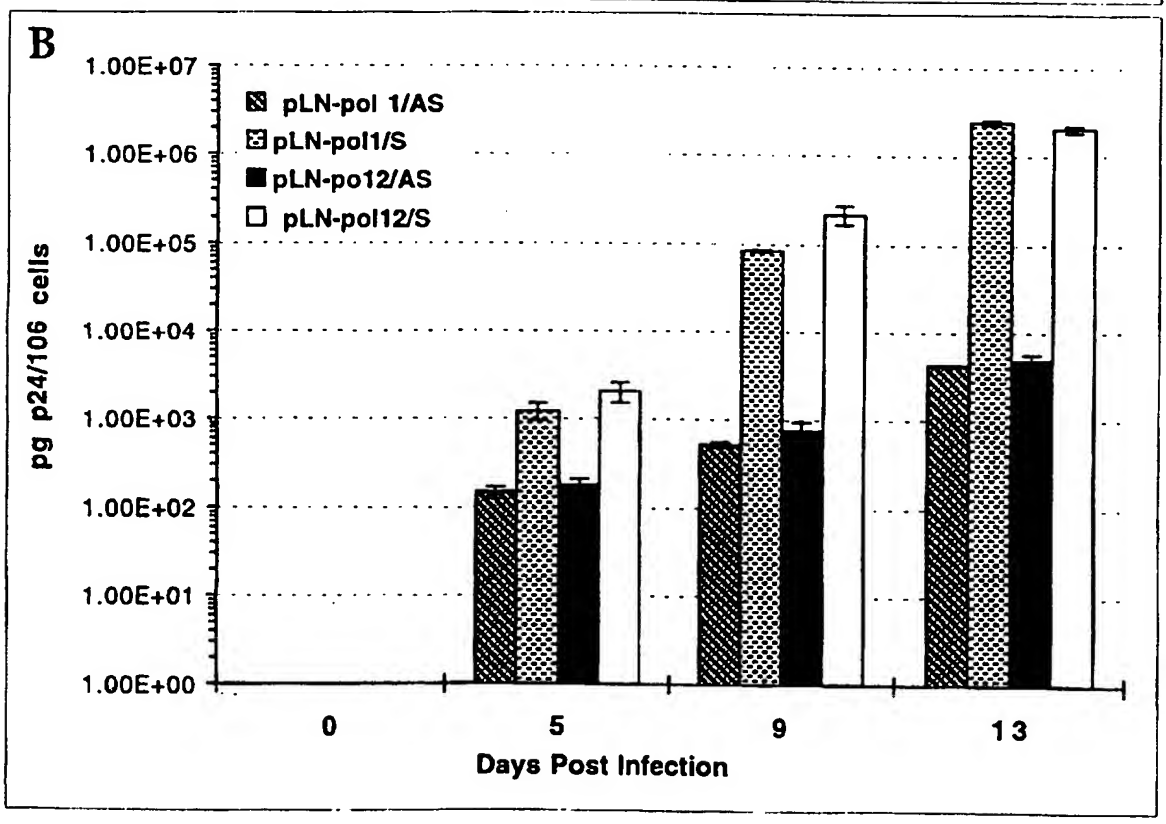
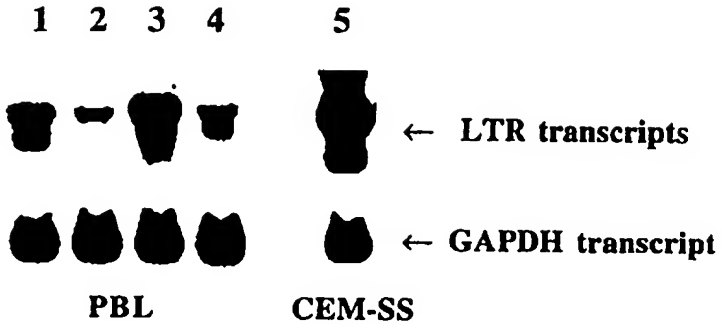
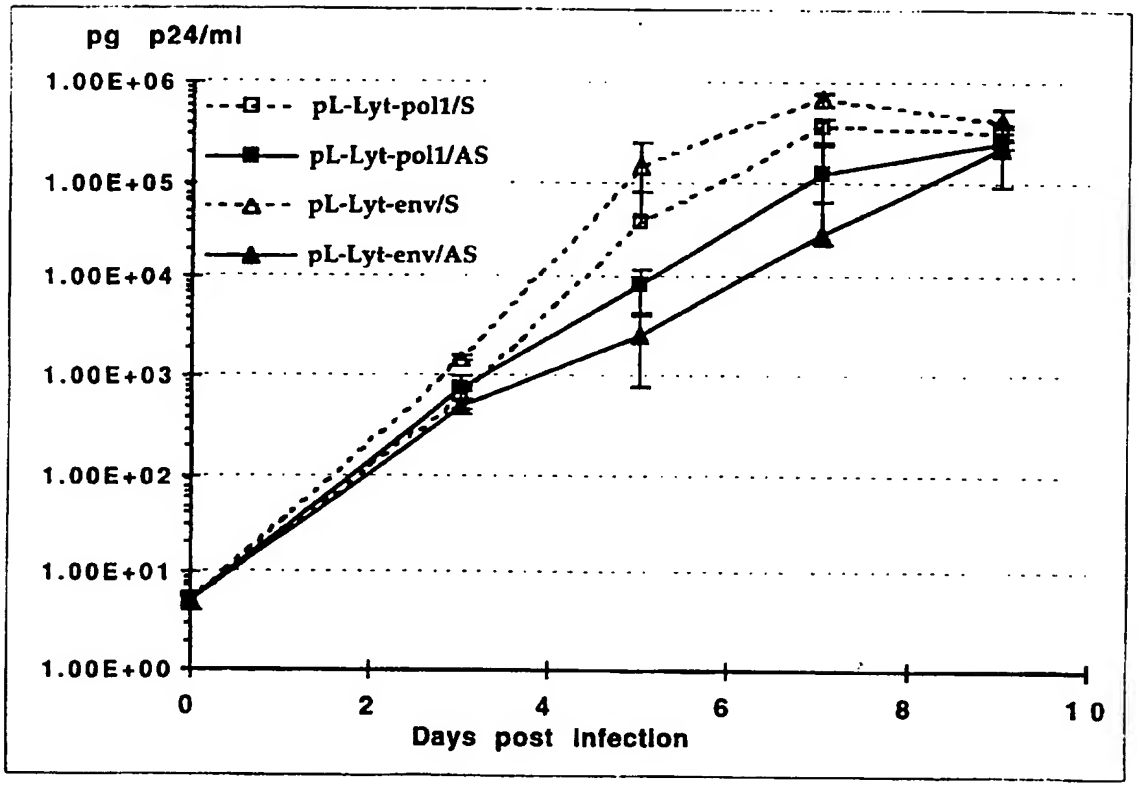

B
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
pg p24/106 cells
pLN-pol 1/AS
pLN-pol1/S
pLN-po12/AS
pLN-pol12/S
0
5
9
13
Days Post Infection

FIGURE 18

A


1 2 3 4 5
← LTR transcripts
← GAPDH transcript
PBL
CEM-SS

B


pg p24/ml
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
pL-Lyt-pol1/S
pL-Lyt-pol1/AS
pL-Lyt-env/S
pL-Lyt-env/AS
0
2
4
6
8
10
Days post infection

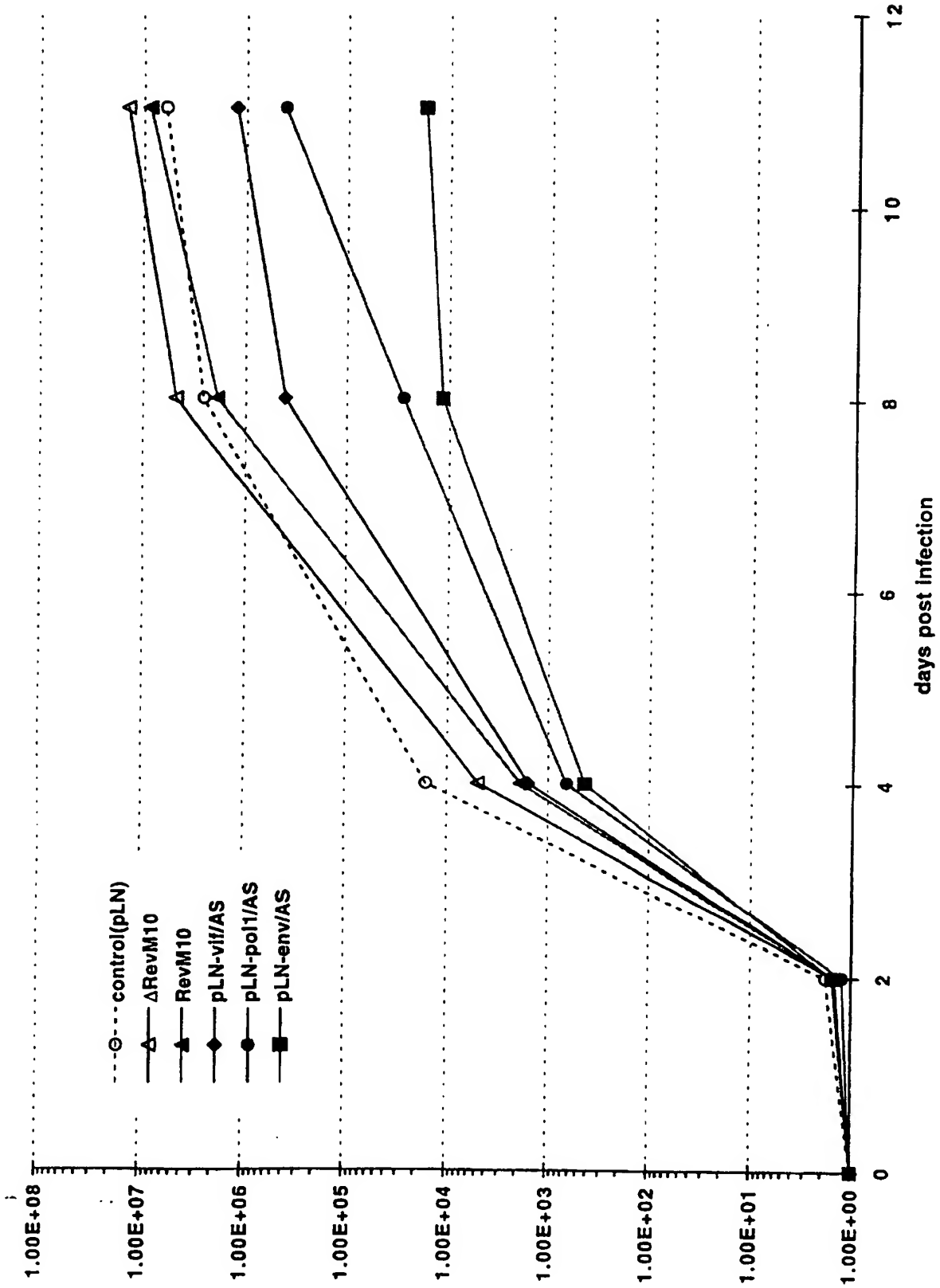

1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
pg p24/10E6 cells
0
2
4
6
8
10
12
days post infection
control(pLN)
ΔRevM10
RevM10
pLN-vif/AS
pLN-pol1/AS
pLN-env/AS

FIGURE 19

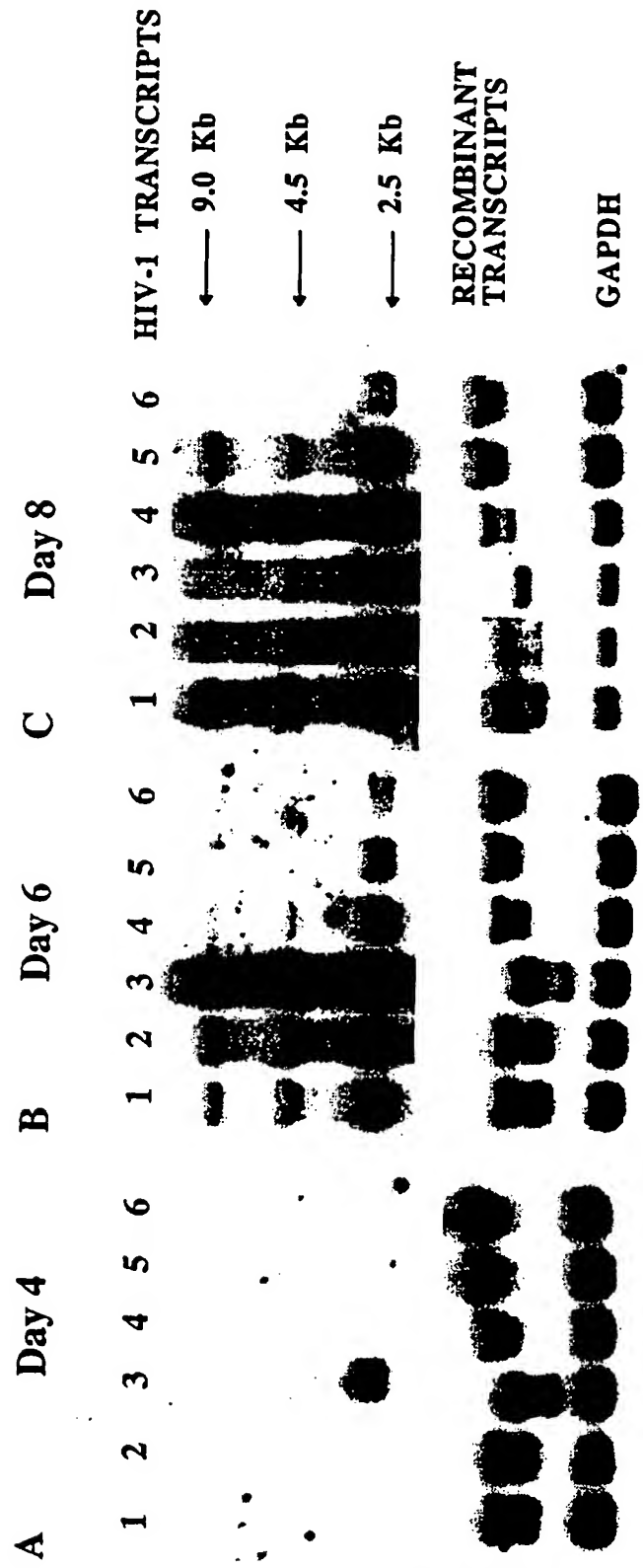

A
Day 4
1 2 3 4 5 6
B
Day 6
1 2 3 4 5 6
C
Day 8
1 2 3 4 5 6
HIV-1 TRANSCRIPTS
9.0 Kb
4.5 Kb
2.5 Kb
RECOMBINANT TRANSCRIPTS
GAPDH

FIGURE 20

A
No. of Cells
Fluorescence Intensity (log_{10})
Control
ΔRevM10
99 %
RevM10
97 %
pLN-vif/AS
89 %
pLN-pol1/AS
27 %
pLN-env/AS
5 %
1
2
3
B
No. of Cells
Fluorescence Intensity (log_{10})
Control
ΔRevM10
52 %
RevM10
27 %
pLN-vif/AS
11 %
pLN-pol1/AS
5 %
pLN-env/AS
3 %
1
2

FIGURE 21

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 15/11, 15/86, A61K 31/70,
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(57) Abstract

Novel antisense sequences to the unspliced or single spliced portions of mRNA transcript from HIV-1 provirus, optionally co-expressed with an inhibitory transdominant mutant HIV-1 protein, are found to be useful in the treatment of HIV-1 infection.

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INTERNATIONAL SEARCH REPORT

International plication No
PCT/EP 97/02952

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 C12N15/86 A61K31/70 C12N15/49 C07K14/16
C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 612 844 A (ORTHO PHARMA CORP) 31 August 1994 see column 2, line 50 - column 3, line 54 see column 5, line 57 - column 6, line 5 see column 8, line 6 - line 28	1-4,6,8, 9
Y	see column 14, line 42 - column 16, line 43 see column 21, line 49 - line 58 see claims see figures 1,8	5,7

Y	WO 90 14427 A (SANDOZ LTD ;UNIV DUKE (US)) 29 November 1990 cited in the application see example 10 see page 74, paragraph 3 - page 75 see claims	5,7

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
12 November 1997	- 2. 12. 97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Internation pplication No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 598 935 A (BAYER AG) 1 June 1994 see the whole document	1,2,6,9
X	RENNEISEN K ET AL: "INHIBITION OF EXPRESSION OF HUMAN IMMUNODEFICIENCY VIRUS-1 IN VITRO BY ANTIBODY-TARGETED LIPOSOMES CONTAINING ANTISENSE RNA TO THE ENV REGION" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 265, no. 27, 25 September 1990, pages 16337-16342, XP000148392 see the whole document	1,2
A	VANDENDRIESSCHE, T. ET AL.: "Inhibition of clinical human immunodeficiency virus (HIV) type 1 isolates in primary CD4+ T lymphocytes by retroviral vectors expressing anti-HIV genes" JOURNAL OF VIROLOGY., vol. 69, July 1995, AMERICAN SOCIETY FOR MICROBIOLOGY US, pages 4045-4052, XP002046632 see the whole document	1-4,6,8
A	WOFFENDIN, C. ET AL.: "Expression of a protective gene prolongs survival of T cells in human immunodeficiency virus-infected patients" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 93, April 1996, WASHINGTON US, pages 2889-2894, XP002046633 cited in the application	

4

INTERNATIONAL SEARCH REPORT

Interna... application No.
PCT/EP 97/02952

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 97/02952

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 10

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 10 is unsearchable by definition, as it is drawn to NOVEL compounds which cannot be found.

Remark : Although claim 8 is directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-9 (all partially)

A nucleic acid capable of generating mRNA annealing to the 1.4 kb fragment from ca. bases 2004 to 3400 of the HIV provirus, a retroviral vector or a cellular composition containing it, and its uses in treatment of HIV infection.

2. Claims: 1-9 (all partially)

A nucleic acid capable of generating mRNA annealing to the 1.2 kb fragment from ca. bases 3400 to 4646 of the HIV provirus, a retroviral vector or a cellular composition containing it, and its uses in treatment of HIV infection.

3. Claims: 1-9 (all partially)

A nucleic acid capable of generating mRNA annealing to the 1.4 kb fragment from ca. bases 6615 to 8053 of the HIV provirus, a retroviral vector or a cellular composition containing it, and its uses in treatment of HIV infection.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internation pplication No
PCT/EP 97/02952

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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